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(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Modified Epimorphin

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(57) 76 Claims

Notice: This application is as filed and may therefore contain an
incomplete specification.



ABSTRACT

Disclosed herein are a modified epimorphin obtained by adding a hydrophilic peptide composed of 5 to 99 amino acids to at least one terminus of a polypeptide containing the functional domain of epimorphin, and a modified epimorphin composed of a polypeptide having a structure that a hydrophobic domain adjacent to the C-terminus of the whole-length epimorphin consisting of a coiled coil domain (1) on the N-terminal side, a functional domain (2) at the center, a coiled coil domain (3) on the C-terminal side and the hydrophobic domain (4) adjacent to the C-terminus has been deleted from the whole-length epimorphin, and at least part of amino acids have been deleted from the terminal side of at least one of the coiled coil domains (1) and (3) as well. The invention also discloses a variant modified epimorphin obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin, wherein the variant maintains the function of the original sequence. The invention further discloses DNAs encoding the modified epimorphin and variant thereof, recombinant vectors containing the DNAs, transformants obtained by introducing the recombinant vectors, and a production method of the modified epimorphin and the variants thereof making use of the transformant.

TITLE OF THE INVENTION:

MODIFIED EPIMORPHIN

FIELD OF THE INVENTION

5 The present invention relates to a modified
epimorphin obtained by modifying epimorphin, which is a
polypeptide existing in mesenchymal cells and controlling
the morphogenesis of epithelial tissue, or a fragment
thereof, and more particularly to a modified epimorphin
10 obtained by modifying epimorphin, which is useful in
elucidating the attack mechanism of diseases caused by the
morphogenetic abnormality of epithelial tissue and
developing diagnosis and medical treatment for these
disease and remedies for wounds, or a fragment thereof
15 while keeping its activity, and variants (variant modified
epimorphin polypeptides) obtained by making partial
substitution, deletion and/or insertion of amino acids in
the amino acid sequence of the modified epimorphin.

 The present invention also relates to DNAs encoding
20 such a modified epimorphin and variants thereof,
recombinant vectors containing the DNAs, transformants
obtained by introducing the recombinant vectors, and a
production method of the modified epimorphin and the
variants thereof making use of the transformant.

25 In the present invention, the term "modified
epimorphin" means a fragment or polypeptide of epimorphin,
which is obtained by modifying the epimorphin or a

fragment thereof.

BACKGROUND OF THE INVENTION

Since the normal organization and morphogenesis of
5 epithelial tissue are under the some control of a factor
originating in mesenchymal cells, and diseases
attributable to the morphogenetic abnormality of the
epithelial tissue may be often caused by the mesenchymal
cells present around the tissue, studies on the mechanism
10 of the mesenchymal cells which support the morphogenesis
of the epithelial tissue have been made for long.
Although studies on the isolation, purification and
structural analysis of a molecule, which controls the
morphogenesis of the epithelial tissue, have been
15 extensively made throughout the world, however, its
substance has been scarcely known under the circumstances
because the object of study is a substance which expresses
under restrictions of time and space in a complicated
system, and so it is difficult to produce a simplified
20 experimental system.

In order to realize the elucidation of diseases
caused by the morphogenetic abnormality of the epithelial
tissue and the attack mechanism thereof, and the
development of medical treatments for these disease, it
25 was an indispensable premise to isolate and purify such a
molecule, which controls the morphogenesis of the
epithelial tissue, and make the structure thereof clear.

Therefore, an important problem in the art was to early achieve the elucidation of the structure of such a molecule, and the like.

In such circumstances, the present inventors
5 recently succeeded in isolation and identification of a molecule (the present inventors termed it "epimorphin") which controls the morphogenesis of the epithelial tissue (Japanese Patent Application Laid-Open No. 25295/1994). Epimorphin is a physiologically active substance
10 comprising, as a core protein, a protein composed of 277 to 289 amino acids and is principally biosynthetically produced by mesenchymal cells.

The present inventors succeeded in determining the amino acid sequences of human and mouse epimorphin
15 molecules. The splicing of their genes has revealed that at least three types exist in respective epimorphin molecules. The human epimorphin molecules include three types, human epimorphin represented by SEQ ID NO. 1, human epimorphin (isoform A) represented by SEQ ID NO. 2 and
20 human epimorphin (isoform B) represented by SEQ ID NO. 3, all shown in SEQUENCE TABLE, which will be described subsequently. The mouse epimorphin molecules include three types, mouse epimorphin represented by SEQ ID NO. 4, mouse epimorphin (isoform A) represented by SEQ ID NO. 5
25 and mouse epimorphin (isoform B) represented by SEQ ID NO. 6, all shown in the SEQUENCE TABLE. The human epimorphin molecules and the mouse epimorphin molecules

have homology of about 90% with each other at the amino acid level. Therefore, they are well conserved even between different animal species.

However, these epimorphin molecules involved a problem that since they firmly bind to a cell membrane at a domain (hereinafter referred to as "the C-terminal hydrophobic domain") adjacent to the C-terminus thereof, which is extremely high in hydrophobic nature, while taking a complex high-order structure in the living body so as to perform their functions, they are extremely difficult to prepare while keeping the activity of epimorphin at a high level. In particular, the epimorphin and the isoform A markedly show such a tendency. When a cell membrane-binding domain exists, it is difficult to secrete epimorphin produced by cultured animal cells into a medium so as to isolate and purify it. The present inventors proposed the preparation of a soluble modified epimorphin by a process of removing the C-terminal hydrophobic domain, and the like (Japanese Patent Application Laid-Open No. 25295/1994). However, these processes have been yet insufficient in the compatibility of the maintenance of high activity with the solubility, and there has hence been a demand for development of a more improved process.

If a modified epimorphin easy to prepare and purify can be obtained while keeping the physiological activity of epimorphin, it is useful in elucidating the attack

mechanism of diseases caused by the morphogenetic abnormality of the epithelial tissue and developing medical treatments for these disease.

5 OBJECT AND SUMMARY OF THE INVENTION

It is an object of the present invention to provide a modified epimorphin which highly keeps the activity of epimorphin and is easy to prepare and purify.

10 The present inventors have analyzed the constitutive amino acids of epimorphin by a computer. As a result, it has been found that epimorphin is roughly divided into four structurally-characteristic domains as illustrated in FIG. 1. More specifically, an epimorphin polypeptide can be divided into a coiled coil domain (1), a functional
15 domain (2), another coiled coil domain (3) and a C-terminal hydrophobic domain (4) from the N-terminal side thereof. The two coiled coil domains can be further divided into some subdomains (for example, heptad repeats and other domains).

20 The present inventors have also found that when a hydrophilic peptide composed of 5 to 99 amino acids is added to the N-terminus and/or C-terminus of a polypeptide containing the functional domain (2) of epimorphin, a modified epimorphin which highly keeps the activity of
25 epimorphin and can be easily purified can be provided.

The present inventors have further found that when at least part of the coiled coil domains (1) and (3) are

deleted from an epimorphin polypeptide from which the C-terminal hydrophobic domain has been deleted, its physiological activity can be enhanced, and consequently, the balance between the physiological activity and the solubility can be adjusted at one's desire. According to this method, a modified epimorphin useful for the development of diagnosis and medical treatment for diseases caused by the morphogenetic abnormality of epithelial tissue, or the development of novel remedies for wounds and the like can be obtained without adversely affecting the high-order structure and activity of epimorphin.

The present invention has been led to completion on the basis of these findings.

According to the present invention, there is thus provided a modified epimorphin obtained by adding a hydrophilic peptide composed of 5 to 99 amino acids to at least one terminus of a polypeptide containing the functional domain of epimorphin.

According to the present invention, there is also provided a modified epimorphin composed of a polypeptide having a structure that a hydrophobic domain adjacent to the C-terminus of the whole-length epimorphin consisting of a coiled coil domain (1) on the N-terminal side, a functional domain (2) at the center, a coiled coil domain (3) on the C-terminal side and the hydrophobic domain (4) adjacent to the C-terminus has been deleted from the

whole-length epimorphin, and at least part of amino acids have been deleted from the terminal side of at least one of the coiled coil domains (1) and (3) as well.

This modified epimorphin may be a variant (variant
5 modified epimorphin) obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence thereof.

According to the present invention, there are further provided DNAs encoding the modified epimorphin and
10 variant thereof, recombinant vectors containing the DNAs, transformants obtained by introducing the recombinant vectors, and a production method of the modified epimorphin and the variants thereof making use of the transformant.

15

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the structural feature of epimorphin and the design of its fragments.

FIG. 2 is an electrophoretogram obtained by
20 preparing the fragments shown in FIG. 1 using Escherichia coli and analyzing them by SDS-PAGE.

FIG. 3 illustrates a result that functional epimorphin fragments has been detected from the fragments shown in FIG. 2 using an antibody binding to the
25 functional site of epimorphin.

FIG. 4 illustrates the design drawing of epimorphin fragments used in Example 4.

FIG. 5 illustrates the structural feature of an epimorphin fragment (1).

FIG. 6 illustrating a relationship between activity and solubility of epimorphin fragments.

5

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Features of the present invention will hereinafter be described in detail.

Epimorphin is a mesenchymal cell membrane molecule
10 essential for the morphogenesis of epithelia in the fetal period and is also considered to participate in the construction of vital tissues. Epimorphin is a membrane protein identified as an antigen molecule recognized by a monoclonal antibody MC-1, which inhibits the normal
15 morphogenesis of epithelia in various fetal tissues [Cell, Vol. 69, p. 471-481 (1992)].

Epimorphin is a protein (molecular weight: about 33 kDa) composed of about 280 amino acids. As human epimorphin molecules, there have been known epimorphin
20 represented by SEQ ID NO. 1, epimorphin isoform A represented by SEQ ID NO. 2 and epimorphin isoform B represented by SEQ ID NO. 3, all shown in the SEQUENCE TABLE. As mouse epimorphin molecules, there have been known epimorphin represented by SEQ ID NO. 4, epimorphin
25 isoform A represented by SEQ ID NO. 5 and epimorphin isoform B represented by SEQ ID NO. 6, all shown in the SEQUENCE TABLE. The mouse epimorphin is useful in, for

example, elucidating the attack mechanism of diseases caused by the morphogenetic abnormality of the epithelial tissue making use of model animals. The human epimorphin is useful in, for example, diagnosing and treating such
5 diseases.

Epimorphin exists in mesenchymal cells around the epithelial tissue and has a function of controlling the morphogenesis of the epithelial tissue, and the like. Such an epimorphin firmly binds to a cell membrane at a C-
10 terminal hydrophobic domain in its molecule while taking a complex stereostructure so as to perform its functions. The predicted product of cDNA of epimorphin is a molecule having a molecular weight of about 33 kDa. It has been already revealed that this molecule forms a plurality of
15 SDS-resistant complexes in the living body, and that of these, an extracellular substance having a molecular weight of about 150 kDa is recognized by the monoclonal antibody MC-1.

According to an analysis by a computer, epimorphin
20 can be roughly divided into 4 domains in structure as illustrated in FIG. 1. The domains other than the C-terminal hydrophobic domain (transmembrane domain) are composed of fragments [coiled coil domains (1) and (3)] on the N-terminal and C-terminal sides, which are easy to
25 take the so-called coiled coil structure with hydrophobic amino acids forming regular lines (heptad repeats), and a central fragment (2) between them, which has substantially

the same size as the former fragments. The coiled coil domains (1) and (3) can be each divided into 4 subfragments including 2 heptad repeats. For reference, the detailed structure of the fragment (1), which is a
5 coiled coil domain, is illustrated in FIG. 5.

According to the results of the study carried out by the present inventors, the central fragment (2) has been found to be a functional domain. The fact that the central fragment (2) of epimorphin is the functional
10 domain can be judged from its reactivity with the monoclonal antibody MC-1 and cellular adhesiveness. As illustrated in FIG. 1, the fragments (1), (2), (3), (12), (13), (23), (123) and (123C: the whole-length epimorphin) were separately prepared with Escherichia coli in
15 accordance with the method known per se in the art to determine their reactivity with the monoclonal antibody MC-1 which binds to the functional site of epimorphin. In particular, the fragments (2) and (23) showed strong reactivity (see Referential Example 1). Since the
20 monoclonal antibody inhibits the activity of the extracellular epimorphin in the living body, this central fragment (2) is considered to be closely related to the activity of epimorphin. The cellular adhesiveness can be determined by, for example, coating dishes (not subjected
25 to a treatment for cell culture) with solutions of the respective fragments in 8 M urea, thoroughly washing the dishes with 8 M urea and PBS (phosphate-buffered saline)

to thinly and evenly spread the fragments on the dishes,
and then seeding epithelial cells on the dishes to
determine their responsibility to the fragments. As a
result, it has been found that the epithelial cells
5 rapidly adhere only to the dish coated with the central
fragment (2). In addition, it has also been found that
the adhering phenomenon of the central fragment (2) to the
epithelial cells is inhibited by the addition of the
monoclonal antibody MC-1. Namely, it has been revealed
10 that the domain of the central fragment (2) directly act
on the cells.

Accordingly, it is apparent that the central
fragment (2) is the functional domain of epimorphin.
Epimorphin and fragments (polypeptides) containing this
15 functional domain are expected to apply to various uses
making good use of the epimorphin activity thereof.
However, some of epimorphin and the functional domain-
containing fragments may be low-soluble or insoluble in
physiological solutions. When a polypeptide containing
20 the functional domain of epimorphin is hardly soluble or
insoluble in physiological solutions, for example, it is
difficult to secrete such a polypeptide produced by
cultured animal cells into a medium so as to isolate and
purify it, and its handling and development to various
25 applications also become difficult.

According to the present invention, a hydrophilic
peptide composed of 5 to 99 amino acids is added to at

least one terminus of a polypeptide containing the functional domain of epimorphin, whereby a modified epimorphin which highly keeps the activity of epimorphin and is soluble in physiological solutions can be obtained.

5 According to the results of the analysis of the amino acid sequence of epimorphin by a computer, in the case of the human epimorphin, the functional domain of epimorphin has been found to be a domain containing an amino acid sequence ranging from the 99th amino acid
10 (phenylalanine) to the 189th amino acid (glutamine) from the N-terminus in common to the epimorphin, the epimorphin isoform A and the epimorphin isoform B. Later, the results of a further investigation as to the human epimorphin have revealed that even a domain containing an
15 amino acid sequence ranging from the 104th amino acid to the 187th amino acid from the N-terminus shows the epimorphin activity. Therefore, the functional domain in the human epimorphin is a domain containing an amino acid sequence ranging from the 99th amino acid to the 189th
20 amino acid, preferably from the 104th amino acid to the 187th amino acid from the N-terminus of the whole-length epimorphin.

 In the case of the mouse epimorphin, the functional domain of epimorphin has been found to be a domain
25 containing an amino acid sequence ranging from the 100th amino acid (cysteine) to the 190th amino acid (glutamine) from the N-terminus in common to the epimorphin, the

epimorphin isoform A and the epimorphin isoform B. Later, the results of a further investigation as to the mouse epimorphin have revealed that even a domain containing an amino acid sequence ranging from the 105th amino acid to the 188th amino acid from the N-terminus shows the epimorphin activity. Therefore, the functional domain in the mouse epimorphin is a domain containing an amino acid sequence ranging from the 100th amino acid to the 190th amino acid, preferably from the 105th amino acid to the 188th amino acid from the N-terminus of the whole-length epimorphin.

<Modified epimorphin obtained by adding a hydrophilic peptide to at least one terminus of a polypeptide containing the functional domain of epimorphin>

No particular limitation is imposed on the polypeptide containing the functional domain of epimorphin so far as it contains the central domain of the epimorphin molecule, which is a functional domain. Such a polypeptide may have any length within limits of epimorphin. For example, a polypeptide composed of the whole-length epimorphin may be included. In general, it is however preferable from the viewpoints of solubility and activity that the C-terminal hydrophobic domain (a domain composed of 23-24 amino acids) be deleted from the whole-length epimorphin. It is also preferable that at least part of amino acids (amino acid residues or peptide) in the coiled coil domain (1) on the N-terminal side,

epimorphin isoform A and the epimorphin isoform B. Later, the results of a further investigation as to the mouse epimorphin have revealed that even a domain containing an amino acid sequence ranging from the 105th amino acid to the 188th amino acid from the N-terminus shows the epimorphin activity. Therefore, the functional domain in the mouse epimorphin is a domain containing an amino acid sequence ranging from the 100th amino acid to the 190th amino acid, preferably from the 105th amino acid to the 188th amino acid from the N-terminus of the whole-length epimorphin.

<Modified epimorphin obtained by adding a hydrophilic peptide to at least one terminus of a polypeptide containing the functional domain of epimorphin>

No particular limitation is imposed on the polypeptide containing the functional domain of epimorphin so far as it contains the central domain of the epimorphin molecule, which is a functional domain. Such a polypeptide may have any length within limits of epimorphin. For example, a polypeptide composed of the whole-length epimorphin may be included. In general, it is however preferable from the viewpoints of solubility and activity that the C-terminal hydrophobic domain (a domain composed of 23-24 amino acids) be deleted from the whole-length epimorphin. It is also preferable that at least part of amino acids (amino acid residues or peptide) in the coiled coil domain (1) on the N-terminal side,

which the domain forms the main cause to mask the activity of the functional domain, be deleted from the whole-length epimorphin according to the kind of the hydrophilic polypeptide to be added. In order to obtain a modified
5 epimorphin high in solubility and easy to handle, it is desirable that at least part of amino acids in the coiled coil domain (3) on the C-terminal side be deleted from the whole-length epimorphin. At least parts of amino acids in the coiled coil domains (1) and (3) on both N-terminal and
10 C-terminal sides may be deleted.

In particular, when amino acids in the coiled coil domain (1) on the N-terminal side of epimorphin are deleted from the N-terminal side, the epimorphin activity of the resulting fragment shows a tendency to enhance. In
15 order to obtain a modified epimorphin higher in activity, therefore, it is preferable that at least part of amino acids in the coiled coil domain (1) on the N-terminal side of epimorphin be deleted from the whole-length epimorphin from the N-terminal side. On the other hand, when amino
20 acids in the coiled coil domain (1) are deleted from the N-terminal side, the solubility of the resulting fragment in physiological solutions shows a tendency to reduce. However, the addition of the hydrophilic peptide to at least one terminus of such a fragment makes it possible to
25 enhance the solubility of the fragment while keeping high activity.

In the case of the human epimorphin, the coiled coil

domain (1) is a domain ranging from the N-terminus to the 103th amino acid in the whole-length epimorphin.

Fragments obtained by deleting 1 to 28 amino acids in such a domain from the N-terminal side can keep good solubility and have enhanced epimorphin activity. Fragments obtained by deleting 29 to 77 amino acids, further, 29 to 103 amino acids in such a domain from the N-terminal side can exhibit higher activity. A domain ranging from the 29th amino acid to the 103th amino acid from the N-terminus contains heptad repeats and is particularly easy to erect a coiled coil structure. Thirty to 98 amino acids from the N-terminal side may be deleted. In any case, it is preferable to delete the C-terminal hydrophobic domain.

In the case of the mouse epimorphin, the coiled coil domain (1) is a domain ranging from the N-terminus to the 104th amino acid in the whole-length epimorphin.

Fragments obtained by deleting 1 to 29 amino acids in such a domain from the N-terminal side can keep good solubility and have enhanced epimorphin activity. Fragments obtained by deleting 30 to 78 amino acids, further, 30 to 104 amino acids in such a domain from the N-terminal side can exhibit higher activity. A domain ranging from the 30th amino acid to the 104th amino acid from the N-terminus contains heptad repeats and is particularly easy to erect a coiled coil structure. Thirty to 99 amino acids from the N-terminal side may be deleted. In any case, it is preferable to delete the C-terminal hydrophobic domain.

The coiled coil domain (3) is a domain obtained by removing the coiled coil domain (1), the functional domain (2) and the C-terminal hydrophobic domain from the whole-length epimorphin and varies according to the species and isoforms of the epimorphin. When the coiled coil domain (3) in the epimorphin other than the isoforms is deleted, it is generally preferable that 25 to 99 amino acids be deleted from the whole-length epimorphin from the C-terminal side.

10 The hydrophilic peptide to be added to the polypeptide containing the functional domain of epimorphin is a hydrophilic peptide of such a size that the solubility of the functional domain of epimorphin is improved and the exhibition of its function is not
15 blocked. Specifically, it is a hydrophilic peptide composed of 5-99 amino acids.

Amino acids can be roughly divided into hydrophilic amino acids and hydrophobic amino acids. The hydrophilic amino acids include glycine (Gly), threonine (Thr),
20 tryptophan (Trp), serine (Ser), tyrosine (Tyr), proline (Pro), histidine (His), glutamic acid (Glu), glutamine (Gln), aspartic acid (Asp), asparagine (Asn), lysine (Lys) and arginine (Arg). The hydrophobic amino acids include
25 isoleucine (Ile), valine (Val), leucine (Leu), phenylalanine (Phe), cysteine (Cys), methionine (Met) and alanine (Ala).

The hydrophilic peptide useful in the practice of

the present invention is a peptide containing bound hydrophilic amino acids in a proportion of, generally, at least 50%, preferably, at least 60%, more preferably, at least 70% based on the number of amino acids. In order to
5 embed the resulting modified epimorphin in vivo, a hydrophilic peptide low in antigenicity may preferably be selected from the viewpoint of safety. In the case where the resulting modified epimorphin is investigated in vitro, a hydrophilic peptide easy to detect may preferably
10 be selected. In any case, it is desirable that the hydrophilic peptide be designed so as to contain such an amino acid sequence that the purification of the resulting modified epimorphin can be performed with ease.

When a hydrophilic peptide containing at least one
15 amino acid sequence selected from the group consisting of, for example, the following amino acid sequences (a) to (h) is used where the in vitro investigation is performed, the resulting modified epimorphin can be detected with good reproducibility because an antibody having high
20 sensitivity to such a hydrophilic peptide is easily available. Two or more of these amino acid sequences may be contained in a hydrophilic peptide.

(a) Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala (i.e.,
YPYDVPDYA),

25 (b) Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Asn
(i.e., EQKLISEEDLN),

(c) Glu-Tyr-Lys-Glu-Glu-Glu-Glu-Lys (i.e.,

EYKEEEEEK),

(d) Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys

(i.e., YTDIEMNRLGK),

(e) Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-

5 Ile-Gly-Lys (i.e., RIQRGPGRAFVTIGK),

(f) Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-Arg

(i.e., ASMTGGQQMGR),

(g) Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp

(i.e., QPELAPEDPED), and

10 (h) Gly-Ala-Pro-Val-Pro-Tyr-Asp-Pro-Leu-Glu-Pro-Arg

(i.e., GAPVPYDPLEPR).

The size and kind of the hydrophilic peptide to be added to the N-terminus and/or the C-terminus of the polypeptide containing the functional domain of epimorphin
15 may be freely chosen for combination as necessary for the intended application of the resulting modified epimorphin. Various kinds of modified epimorphin obtained by separately adding different hydrophilic peptides may be suitably mixed before use. If for example, at least five
20 histidine (His) residues are consecutively contained in the hydrophilic peptide, the modified epimorphin prepared from this can be purified by one step through a column in which nickel has been fixed.

The hydrophilic peptide is added to the N-terminus,
25 C-terminus or both termini of the polypeptide containing the functional domain of epimorphin. Whether the hydrophilic peptide is added to one terminus or to both

termini can be suitably determined in view of the solubility of the polypeptide containing the functional domain of epimorphin in physiological solutions, the degree of epimorphin activity of the polypeptide, the kind
5 of the hydrophilic peptide, and the like.

If the polypeptide containing the functional domain of epimorphin is composed of the whole-length epimorphin, the hydrophilic peptide may preferably be added to both termini thereof. If the polypeptide containing the
10 functional domain of epimorphin is a fragment obtained by deleting the C-terminal hydrophobic domain, the hydrophilic peptide may preferably be added to either the N-terminal or both termini.

If the polypeptide containing the functional domain
15 of epimorphin is a fragment obtained by deleting at least part of amino acids in the coiled coil domain (1) from the whole-length epimorphin from the N-terminal side, the hydrophilic peptide may preferably be added to either the N-terminal or both termini in order to avoid reduction in
20 the solubility. Even if the polypeptide containing the functional domain of epimorphin is a fragment obtained by deleting at least part of amino acids in the coiled coil domain (1) from the whole-length epimorphin from the N-terminal side and moreover deleting the C-terminal
25 hydrophobic domain, the hydrophilic peptide may also be preferably added to either the N-terminal or both termini.

If the polypeptide containing the functional domain

of epimorphin is a fragment obtained by deleting the C-terminal hydrophobic domain and at least part of amino acids in the coiled coil domain (3) on the C-terminal side from the whole-length epimorphin, the hydrophilic peptide
5 may preferably be added to either the C-terminal or both termini.

As a method of adding the hydrophilic peptide to one or both termini of the polypeptide containing the functional domain of epimorphin, there may be used various
10 techniques such as biochemical techniques and gene engineering techniques. As an example of a method making use of a biochemical technique, may be mentioned a process in which an epimorphin molecule itself is chemically or physically cleaved to obtain a fragment containing the
15 functional domain, the fragment is purified, a hydrophilic peptide is then bound to the N-terminus and/or C-terminus of the fragment making use of a chemical reaction.

As an example of a method making use of a gene engineering technique, may be mentioned a process in which
20 a part of a gene encoding epimorphin and a gene encoding a hydrophilic peptide to be added are integrated into an appropriate vector to express a modified epimorphin in a host. As a specific technique, there may be used, for example, the following simple technique.

25 cDNA (A) encoding a fragment containing the functional domain of epimorphin is first synthesized by polymerase chain reaction (PCR). On the other hand,

single-stranded DNAs (B and C), in which DNA encoding a hydrophilic peptide to be added to the N-terminus and/or C-terminus of the above fragment are joined to 10 to 20 bases on the N-terminal and C-terminal sides of the fragment containing the functional domain of epimorphin, respectively, are separately prepared by DNA synthesizer. A double-stranded DNA encoding the intended modified epimorphin is then obtained by PCR making use of the cDNA (A) as a template, and the DNAs (B) and (C) as primers. After the thus-obtained DNA is integrated into a proper site of an expression vector, gene transfer is performed into a proper host such as Escherichia coli or animal cells. The gene-transferred host is cultured under suitable conditions to induce the expression of the transgene, thereby preparing the modified epimorphin.

Since the modified epimorphin according to the present invention has a nature that it is easily dissolved in a physiological solution, its mass production and purification can be performed with ease. In addition, such a modified epimorphin highly keeps the epimorphin activity. The thus-obtained modified epimorphin is purified by an appropriate method, for example, affinity chromatography or the like, and then used as it is. When a plurality of modified epimorphin molecules is chemically crosslinked to each other to form a complex, however, higher activity may be exhibited in some cases. In this case, the crosslinking may be conducted with ease by an

irradiation treatment with ultraviolet (UV) rays or a chemical modification making use of a chemical substance such as glutaraldehyde or DSS (disuccinimidyl suberate). These crosslinking treatments may make the solubility of the resulting complex in physiological solutions lowered. For example, when the modified epimorphin is coated on a base material such as a plastic or metal to use, it is therefore necessary to give such considerations that the crosslinking treatment is performed after the coating of the modified epimorphin.

For example, the counting of the number of attached cells and the reactivity with the monoclonal antibody MC-1 may be used as indices to the evaluation of activity of the modified epimorphin. As examples of the method of counting the number of attached cells, may be mentioned the following methods. The modified epimorphin is suspended in a 8 M urea/Lysis buffer, and this suspension is coated on a suspension culture dish and then dried. Thereafter, the thus-coated dish is washed once with the 8 M urea/Lysis buffer and then 5 times with PBS. Cultured cells CH3/10T1/2 clone 8 (product of Dainippon Pharmaceutical Co., Ltd.) suspended in a D-MEM/F-12 medium (SIGMA D8900) added with 20 mg/ml of BSA (Bovine Serum Albumin) were then seeded on the coated dish. After left over for a predetermined period of time (for example, 4 hours), the dish is washed 3 times with PBS, and the cells are then lysed with 0.5 N NaOH, followed by recovery of

the resultant solution to measure the amount of DNA by a spectrophotometer, thereby counting the number of cells attached to the dish. Alternatively, an untreated polystyrene surface is coated with the modified epimorphin (10 μcm^2), and thoroughly washed with PBS. Thereafter, MDCKII cells (model of epithelial cells) suspended in a serum-free medium (Dulbecco MEM) containing 20 mg/ml of BSA are seeded on the surface. After left over for a predetermined period of time (for example, 8 hours), the number of cells attached to the surface is determined. These methods can also be applied to epimorphin and fragments of epimorphin.

As an index to the evaluation of solubility of the modified epimorphin, there may be used the amount of a fraction soluble in a physiological solution such as PBS. More specifically, the solubility may be evaluated by an amount of a soluble fraction when 1 mg of a protein is dissolved in 1 ml of PBS at 37°C. Therefore, the fact that 85% of the protein is soluble means that 0.85 mg of the protein is dissolved when 1 ml of PBS is added to 1 mg of the protein to mix them.

<Modified epimorphin composed of a polypeptide having a structure that at least part of amino acids have been deleted from the terminal side of at least one of the coiled coil domains (1) and (3)>

The central fragment (2) of epimorphin is a functional domain of epimorphin. Fragments containing the

functional domain (2) are expected to apply to various uses making good use of the epimorphin activity thereof. However, the polypeptides containing the functional domain (2) may be insoluble or hardly soluble in physiological solutions, or low in activity. When the C-terminal hydrophobic domain is deleted from the whole-length epimorphin, a polypeptide soluble in the physiological solutions can be obtained. However, such a polypeptide is low in activity.

10 As a result of a detailed analysis as to the activity and solubility of the fragments of epimorphin, it has been found that the fragment (123) is soluble, but extremely low in activity, and the fragment (23) is insoluble, but high in activity by contraries. From this, 15 the present inventors have built up the following hypothesis as to the work of the fragment (1). Namely, the fragment (1) negatively works on activity due to the action to mask the functional domain, but works on the solubility to enhance it owing to the action to change the 20 high-order structure of the fragment (23).

 Thus, the present inventors have produced a 2M fragment obtained by deleting 28 amino acids (in the case of human epimorphin) or 29 amino acids (in the case of mouse epimorphin) from the N-terminal side of the fragment 25 (123), a 3M fragment obtained by deleting 77 amino acids (in the case of human epimorphin) or 78 amino acids (in the case of mouse epimorphin) from the N-terminal side of

the fragment (123), and a fragment (23) as illustrated in FIG. 6 to analyze their properties. As a result, it has been revealed that the epimorphin fragments have a relationship that the activity increases, but on the other
5 hand, the solubility decreases as the number of amino acids deleted from the N-terminal side is increased as illustrated in FIG. 6 (see the right of the drawing). It has hence been revealed that the activity and solubility of epimorphin polypeptides can be controlled by increasing
10 or decreasing the number of amino acids to be deleted from the N-terminal side.

According to the present invention, as described above, there can be provided modified epimorphin polypeptides the activity and solubility of which can be
15 controlled for the purpose of developing diagnosis and medical treatment for diseases caused by the morphogenetic abnormality of epithelial tissue, or novel remedies for wounds and the like.

More specifically, in the case where one attaches
20 much importance to the activity, it is only necessary to produce a fragment with the number of amino acids deleted from the N-terminal side increased. In the case of human epimorphin, it is only necessary to produce a polypeptide having a structure that 78 to 103 amino acids, preferably,
25 91 to 103 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in activity. In the case of mouse epimorphin, it is

only necessary to produce a polypeptide having a structure that 79 to 104 amino acids, preferably, 92 to 104 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in activity. For
5 example, the fragment (23) may preferably be used.

In the case where one attaches much importance to the solubility, it is only necessary to produce a fragment with the number of amino acids deleted from the N-terminal side decreased. In the case of human epimorphin, it is
10 only necessary to produce a polypeptide having a structure that 1 to 28 amino acids, preferably, 14 to 28 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in solubility. In the case of mouse epimorphin, it is only necessary to produce
15 a polypeptide having a structure that 1 to 29 amino acids, preferably, 14 to 29 amino acids have been deleted from the N-terminal side in order to obtain a modified epimorphin high in activity. For example, the fragment (2M) may preferably be used.

20 In the case where a fragment well balanced between the activity and the solubility is intended to use, it is only necessary to produce a fragment in which the number of amino acid deleted from the N-terminal side is intermediate between the above ranges. In the case of
25 human epimorphin, it is only necessary to produce a polypeptide having a structure that 29 to 77 amino acids, preferably, 61 to 77 amino acids have been deleted from

the N-terminal side. In the case of mouse epimorphin, it is only necessary to produce a polypeptide having a structure that 30 to 78 amino acids, preferably, 62 to 78 amino acids have been deleted from the N-terminal side.

5 For example, the fragment (3M) may preferably be used.

As described above, the modified epimorphin according to the present may be used properly with its structure changed according to the purpose and application intended. Therefore, the use of the modified epimorphin
10 according to the present invention allows the development of diagnosis and medical treatment for diseases caused by the morphogenetic abnormality of epithelial tissue, or the development of novel remedies for wounds and the like to more effectively progress. Epimorphin is a protein
15 composed of about 280 amino acids. The mouse epimorphin is useful in, for example, elucidating the attack mechanism of diseases caused by the morphogenetic abnormality of the epithelial tissue making use of model animals. The human epimorphin is useful in, for example,
20 diagnosing and treating such diseases. These molecules exist in mesenchymal cells around the epithelial tissue and have a function of controlling the morphogenesis of the epithelial tissue, and the like.

As a method of deleting amino acids from the N-
25 terminal side of epimorphin, there may be used various techniques such as biochemical techniques and gene engineering techniques. As an example of a method making

use of a biochemical technique, may be mentioned a process in which an epimorphin molecule is chemically or physically cleaved to obtain the above-described fragments. As an example of a method making use of a gene engineering technique, may be mentioned a process in which
5 cDNA encoding each of the fragments of epimorphin is integrated into an appropriate vector to express the fragment in a host. As a specific technique, there may be used, for example, the following simple technique.

10 cDNA (A) encoding the whole-length epimorphin is first synthesized by polymerase chain reaction (PCR). On the other hand, single-stranded DNAs (B and C), in which 10 to 20 bases on the N-terminal and C-terminal sides of the epimorphin fragment to be prepared are joined to one
15 another, respectively, are separately prepared by a DNA synthesizer. A double-stranded DNA encoding the intended modified epimorphin is then obtained by PCR making use of the cDNA (A) as a template, and the DNAs (B) and (C) as primers.

20 The thus-obtained double-stranded DNA is integrated into a vector having a structure capable of expressing the DNA, for example, pET3C (RIKEN DNA Bank RDB519) to prepare a recombinant vector. This recombinant vector is then introduced into a proper host, for example, BL21 (RIKEN
25 DNA Bank RDB022) to obtain a transformant. After propagating this transformant in a large amount, a treatment for inducing expression, for example, the

addition of IPTG to a medium so as to give a final concentration of 1 mM, is given to obtain the intended modified epimorphin.

5 DNA sequences encoding the fragments (2M), (3M), (23) and (123) of the human epimorphin are shown in the SEQUENCE TABLE. SEQ ID NOS. 7, 8, 9 and 10 are DNA sequences of the fragments (2M), (3M), (23) and (123) of the human epimorphin, respectively.

10 In the SEQUENCE TABLE, are shown DNA sequences encoding the fragments (2M), (3M), (23) and (123) of the mouse epimorphin. SEQ ID NOS. 11, 12, 13 and 14 are DNA sequences of the fragments (2M), (3M), (23) and (123) of the mouse epimorphin, respectively.

15 Amino acid sequences of the fragments (2M), (3M) and (23) of the human epimorphin are shown in the SEQUENCE TABLE. SEQ ID NOS. 15, 16 and 17 are amino acid sequences of the fragments (2M), (3M) and (23) of the human epimorphin, respectively.

20 Amino acid sequences of the fragments (2M), (3M) and (23) of the mouse epimorphin are shown in the SEQUENCE TABLE. SEQ ID NOS. 18, 19 and 20 are amino acid sequences of the fragments (2M), (3M) and (23) of the mouse epimorphin, respectively.

25 The modified epimorphin according to the present invention keeps the activity inherent in epimorphin at a high level, and is hence used directly in various applications including medical care, for example,

treatments of various tissues for burns or scalds, or after surgery, and artificial organs. Besides, it may also be used at a low concentration as a component for cosmetics, hair growth stimulants and the like as it is.

5 The modified epimorphin according to the present invention may be a variant obtained by making partial substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin so far as it substantially keeps the epimorphin activity. The
10 partial substitution, deletion and insertion of amino acids may be made either singly or in any combination thereof. The site of the amino acid sequence (the variable site), at which the partial substitution, deletion or insertion of amino acids is made, is generally
15 in the amino acid sequence of a polypeptide containing the functional domain (2) of epimorphin. This variable site may be in the amino acid sequence of the functional domain (2) of epimorphin. Such a variant itself may be easily produced by the method known per se in the art. Namely,
20 the technique itself, in which partial substitution, deletion or insertion is made in the amino acid sequence of a protein to obtain a variant of the protein, is generally known. For example, recombinant PCR ("PCR Protocols" 155-160, Harcourt Brace Javanovich Japan Inc.
25 1991) or producing method of recombinant gene with PCR ("Experimental Medicine" Vol. 8, No. 9, 63-67, Yodosha Co., Ltd. 1990). The variant modified epimorphin

according to the present invention may preferably substantially keep the functions inherent in the modified epimorphin, such as good cellular adhesiveness.

5 ADVANTAGES OF THE INVENTION

According to the present invention, the modified epimorphin which highly keeps the activity of epimorphin and are easy to prepare and purify is provided by adding a hydrophilic peptide to a polypeptide containing the
10 functional domain (2) of epimorphin. Besides, according to the present invention, the modified epimorphin excellent in both activity and solubility is provided by deleting at least part of amino acids from the terminal side of at least one of the coiled coil domains (1) and
15 (3). More specifically, the modified epimorphin which is soluble and far excellent in activity, or the modified epimorphin which is active and far excellent in solubility is provided. According to the present invention, there are further provided variants obtained by making partial
20 substitution, deletion and/or insertion of amino acids in the amino acid sequence of the modified epimorphin. The modified epimorphin and variants thereof according to the present invention are soluble in physiological solutions and can hence be mass-produced and is easy to purify. The
25 modified epimorphin and variants thereof of this invention keep the activity inherent in epimorphin at a high level, and is hence used directly in various applications

including medical care, for example, treatments of various tissues for burns or scalds, or after surgery, and artificial organs. Besides, they are useful as a component for cosmetics, hair growth stimulants and the
5 like.

The proper use of the modified epimorphin and variants thereof according to the intended application in the research and medical fields allows the development of diagnosis and medical treatment for diseases caused by the
10 morphogenetic abnormality of epithelial tissue, or the development of novel remedies for wounds and the like to more effectively progress.

EMBODIMENTS OF THE INVENTION

15 The present invention will hereinafter be described in more detail by the following examples.

Referential Example 1:

The analysis by a computer revealed that the constitutive amino acids of epimorphin are divided into
20 four structurally-characteristic domains as illustrated in FIG. 1. Incidentally, the coiled coil domains (1) and (3) can each be further divided into four subfragments.

Thus, with respect to mouse epimorphin (polypeptide composed of 289 amino acids in the whole length)
25 represented by SEQ ID NO. 4 of the SEQUENCE TABLE, peptide fragments corresponding to the respective domains were first designed as illustrated in FIG. 1 (fragments

indicated by 1, 2, 3, 12, 13, 23, 123 and 123C at lower rows in FIG. 1).

Respective cDNAs encoding the peptide fragments were separately prepared by PCR making use of the whole-length
5 cDNA of the mouse epimorphin as a template, and separately inserted into the NdeI-BamHI site of an expression vector PET3C [Gene, Vol. 56, 125-135 (1987)]. The thus-obtained vector/fragment cDNAs were separately introduced into Escherichia coli to induce them with IPTG (1 mM, 2 hours),
10 thereby producing the respective epimorphin fragments in the Escherichia coli. Total proteins in the Escherichia coli were analyzed by SDS-PAGE. As a result, it was found that all the epimorphin fragments were produced in substantially equal amounts (see FIG. 2). These fragments
15 were then electrically transferred to a nitrocellulose membrane to determine the functional site of epimorphin with a monoclonal antibody MC-1 [Cell, Vol. 69, 471-481 (1992)] which binds to the functional site. The domain situated at the center of the epimorphin molecule was
20 found to correspond to the functional site (see FIG. 3).

Even in the fragments containing the functional domain, those containing a sequence on the N-terminal side or a hydrophobic domain composed of 23 to 24 amino acids adjacent to the C-terminus at the same time were found to
25 be low in the reactivity to the antibody (namely, the functional site was masked). Therefore, the function was not smoothly exhibited (compare the fragments 12 and 123

with the fragment 2 and 123C in FIG. 3, respectively).
The fragment indicated by 23 in FIG. 3 was extracted from
the Escherichia coli to purify it. As a result, it was
revealed that when amino acids up to 99th amino acid from
5 the C-terminus are deleted from such a fragment, the
solubility of epimorphin in a physiological solution (PBS)
is significantly increased (the insolubility of at least
95% turned to the solubility of 40%). Namely, the
fragment 23 is hardly soluble compared with the fragment
10 2. However, its solubility is increased by deleting the
fragment 3.

On the basis of the results thus obtained, it was
found that it is only necessary to contain an amino acid
sequence ranging from the 100th amino acid to the 190th
15 amino acid from the N-terminus in order to exhibit the
function of epimorphin, that its function is gradually
improved by successively deleting 30 to 99 amino acids
from the N-terminal side, and that its solubility is
gradually improved by successively deleting 25 to 99 amino
20 acids from the C-terminal side.

Example 1:

A cDNA encoding a fragment (amino acid sequence
ranging from 100th to 190th amino acids from the N-
terminus) containing the functional domain of mouse
25 epimorphin was prepared by PCR in the same manner as in
Referential Example 1 and inserted into the NdeI or BamHI
site of a vector PET3C [Gene, Vol. 56, 125-135 (1987)].

The thus-obtained vector/fragment cDNA was then introduced into Escherichia coli to produce polypeptides in which a peptide ASMTGGQQMGR was added as a hydrophilic peptide to the C-terminus or N-terminus of the above fragment.

5 The thus-obtained polypeptides were purified. As a result, all the polypeptides had a solubility of about 50% (namely, the solubility was improved by 10%). Even in the cases where peptides YPYDVDPDYA, EQKLISEEDLN, EYKEEEEK, YTDIEMNRLGK, RIQRGPGRAFTIGK, QPELAPEDPED and GAPVPYDPLEPR
10 were separately added in accordance with the same procedure as described above, the solubility was recognized to be improved by about 5-15%. The polypeptides thus obtained were all able to be detected with good reproducibility by commercially-available
15 specific antibodies.

On the other hand, a cDNA produced by selecting, as one of primers, a product obtained by connecting a single-stranded DNA encoding the sequence (ASMTGGQQMGR) composed of 11 amino acids to a positive-chain DNA encoding an
20 amino acid sequence ranging from the 100th to 115th amino acids of epimorphin was inserted into the NdeI site of the above vector to conduct the same procedure as described above, thereby producing polypeptides in which the eleven amino acids were added to both C-terminus and N-terminus
25 of the epimorphin functional domain-containing fragment. The thus-obtained polypeptides had a solubility of at least 85% in PBS.

MDCKII cells (model of epithelial cells) were suspended in a medium (Dulbecco MEM added with 10% serum) and then incubated for 8 hours in the presence of an equiamount of the soluble fraction of the epimorphin
5 fragment. The thus-incubated cells were washed and then electrophoresed to detect the epimorphin fragments bound to the cells using an anti-ASMTGGQQMGR antibody (anti-T7-tag monoclonal antibody, product of Nobagen Co.). As a result, it was revealed that the epimorphin functional
10 domain-containing fragment (modified epimorphin fragment) to both C-terminus and N-terminus of which the hydrophilic peptides composed of the eleven amino acids were added particularly firmly bound to the cells and then incorporated therein.

15 Example 2:

A single-stranded DNA (99 bases) encoding the 11 amino acid sequence (ASMTGGQQMGR) repeatedly 3 times was produced by a DNA synthesizer and then converted to a double-stranded DNA with DNA polymerase. This DNA was
20 inserted repeatedly several times into PET3C vectors in which DNAs respectively encoding the epimorphin functional domain-containing fragments obtained in Example 1, to the C-terminus or N-terminus of which the amino acids had been added, had been separately inserted, thereby producing
25 epimorphin functional domain-containing fragments, to both C-terminus and N-terminus of which amino acids of various lengths (11, 33, 66, 99, 44, 76 and 110) were added, in

Escherichia coli.

The function of the fragments thus obtained was evaluated with the MDCKII cells in the same manner as in Example 1. As a result, it was found that good cellular
5 adhesiveness is recognized in the cases where the amino acids are added up to the number of 99 to both N- and C-termini, but the cellular adhesiveness tends to lower when the length of the amino acids added becomes longer than 99.

10 Example 3:

Polypeptides in which 3 to 15 consecutive His residues were added to the N-terminus of a fragment composed of the functional domain (amino acid sequence ranging from the 105th to 188th amino acids from the N-
15 terminus) of mouse epimorphin were first produced in Escherichia coli in the same manner as in Example 1. The Escherichia coli strain thus obtained was lysed in 8 M urea to attempt one-step purification through a Ni-agarose column. As a result, it was possible to purify fragments
20 without containing any other contaminant when a hydrophilic peptide containing at least 5 consecutive His residues was added.

Example 4:

Polypeptides in which 6 consecutive His residues
25 were added to the N-termini of epimorphin fragments of various lengths containing the functional domain of mouse epimorphin were first produced in the same manner as in

Example 1. The respective fragments are shown in FIG. 4.

In FIG. 4, the fragments are as follows:

123: a fragment ranging from the N-terminus to right before the C-terminal hydrophobic domain;

5 2M: a fragment ranging from the 30th amino acid from the N-terminus to right before the C-terminal hydrophobic domain;

3M: a fragment ranging from the 79th amino acid from the N-terminus to right before the C-terminal
10 hydrophobic domain;

23: a fragment ranging from the 105th amino acid from the N-terminus to right before the C-terminal hydrophobic domain;

2: a fragment ranging from the 105th amino acid to
15 the 188th amino acid from the N-terminus; and

13C: a fragment obtained by deleting the fragment 2 from the whole-length epimorphin.

These fragments were then purified through a Ni-agarose column and separately coated on culture dishes,
20 and angioendothelial cells were then seeded on the dishes. As a result, the angioendothelial cells rapidly adhered to all the peptides containing the functional domain (the fragment 2). However, no cell adhesion was observed on the fragment obtained by deleting only the functional
25 domain from the whole-length epimorphin.

The amounts of factors secreted by the cells adhered were determined. As a result, it was found that the

fragment obtained by adding the His residues to the fragment 123 is highest in activity.

Example 5:

An internal ear of a rabbit was first wounded in a size 6 mm across and 1 mm deep by a punch. This wound was treated with 1-10 μ g of the modified epimorphin fragment (peptide containing the fragment 123) obtained in Example 4 and then taped. After 1 week, the tape was taken out of the wounded site to prepare slices from this wounded site, thereby determining reepithelialization rate, granulation rate and the number of neoplastic vessels. As a result, this modified epimorphin fragment was found to be useful in healing wounds. The use of the modified epimorphin fragment (peptide containing the fragment 3M) obtained in Example 4 gave the same results as described above. Therefore, it is understood that the modified epimorphin fragments containing the functional domain are useful in healing wounds.

Example 6:

The modified epimorphin fragment produced in Example 1, in which the hydrophilic peptide composed of the eleven amino acids (ASMTGGQQMGR) had been added to both termini thereof, was coated on two positions of an untreated polystyrene surface in density of 10 μ g/cm². One of the coated portions was crosslinked with DSS (disuccinimidyl suberate). After the thus-coated surface was thoroughly washed with PBS, MDCKII cells suspended in a serum-free

medium (Dulbecco MEM) containing 20 mg/ml of BSA were seeded on the surface. After 2 hours and 8 hours, the number of cells attached to the fragments was determined. As a result, it was revealed that MDCKII rapidly and strongly bind to the crosslinked portion (see Table 1). Incidentally, the coating fragments on the polystyrene surface were dissolved in a sample buffer containing SDS without seeding the cells to collect them, thereby analyzing them by electrophoresis. As a result, it was confirmed that only the fragment subjected to the DSS treatment was polymerized.

Table 1

15		Modified epimorphin obtained by adding the hydrophilic peptide composed of 11 amino acids to both termini of the epimorphin functional domain-containing fragment	Modified epimorphin obtained by adding the hydrophilic peptide composed of 11 amino acids to both termini of the epimorphin functional domain-containing fragment and crosslinking with DSS
20	Number of cells attached after 2 hours	10%	70%
	Number of cells attached after 8 hours	85%	90%

(Note) The number of attached cells (%) is a value calculated out on the basis of the whole number (100%) of cells seeded.

Example 7:

Production of modified epimorphin

(1) Cell:

A mesenchymal cell strain expressing epimorphin, for example, CH3/10T1/2 clone 8 (Code No. 08-226, product of Dainippon Pharmaceutical Co., Ltd.) was purchased to culture it in accordance with the description thereof.

(2) Preparation of RNA:

A TRIzol Reagent (Cat. No. 15596-026) produced by Lifetec Oriental K.K. was used in the preparation of RNA. The RNA was prepared in accordance with a protocol attached to the product.

After the preparation, the RNA was treated with DNase I (Cat. No. 8068SA, amplification grade) produced by Lifetec Oriental K.K. in accordance with a protocol attached to the product.

(3) Preparation of epimorphin cDNA by RT-PCR:

On the basis of the RNA thus prepared, a reverse transcription was performed with an RNA PCR kit (Cat. No. R012) available from Takara Shuzo Co., Ltd. in accordance with a protocol attached to the product.

In order to amplify only cDNA of epimorphin, both upstream primer and downstream primer (5'ATGCGGGACCGGCTG3' and 5'TCATTTGCCAACCGA3') specific for epimorphin were then used to perform PCR in accordance with a protocol attached to the product, thereby obtain epimorphin cDNA. The preparation of the upstream and downstream primers specific for epimorphin was entrusted to Bex K.K.

(4) Preparation of fragments:

With respect to cDNAs encoding the fragments (2M), (3M), (23) and (123) of human epimorphin, base sequences of primers specific for the respective fragments were
5 determined from SEQ ID NO. 7 (human 2M), SEQ ID NO. 8 (human 3M), SEQ ID NO. 9 (human 23), SEQ ID NO. 10 (human 123), and primers with a restriction enzyme NdeI site (5'CATATG3') and a restriction enzyme NheI site (5'GCTAGC3') tagged on the 5' side of the upstream primer
10 and the 5' side of the downstream primer, respectively, were purchased from Bex K.K.

Similarly, with respect to cDNAs encoding the fragments (2M), (3M), (23) and (123) of mouse epimorphin, base sequences of primers specific for the respective
15 fragments were determined from SEQ ID NO. 11 (mouse 2M), SEQ ID NO. 12 (mouse 3M), SEQ ID NO. 13 (mouse 23), SEQ ID NO. 14 (mouse 123), and primers with a restriction enzyme NdeI site (5'CATATG3') and a restriction enzyme NheI site (5'GCTAGC3') tagged on the 5' side of the upstream primer
20 and the 5' side of the downstream primer, respectively, were purchased from Bex K.K.

The respective fragments were obtained by PCR making use of respective pairs of the primers. The PCR was performed with a Takara Taq (Cat. No. R001A) available
25 from Takara Shuzo Co., Ltd. in accordance with a protocol attached to the product.

(5) Subcloning:

Each of the double-stranded DNAs thus obtained was integrated into an expression vector, for example, a vector derived from pET3C (RIKEN DNA Bank RDB519) by deleting a domain lying between two EcoRV sites thereof, in accordance with the process described in "Laboratory-Manual Gene Engineering", 111-114 (1988), published by Maruzen Co., Ltd., thereby producing a recombinant vector.

This recombinant vector was then introduced into a host, BL21 (RIKEN DNA Bank RDB022), in accordance with the Hanahan method described in "Laboratory-Manual Gene Engineering", 108-109, thereby obtaining transformants.

(6) Screening:

Colonies grown on an LB plate (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 1% NaCl, 1.5% Bacto-agar) containing 50 µg/ml of ampicillin were selected to conduct the primary screening of the transformants.

In order to finally identify the transformants having the recombinant vector, the recombinant vector contained in the transformant was used as a template to perform PCR using the upstream and downstream primers specific for the modified epimorphin intended to ascertain whether the epimorphin cDNAs were present or not (at this point of time, 9 clones out of 10 clones contained the cDNAs).

After the transformants thus obtained were propagated in large amounts by shaking culture at 37°C on a liquid LB medium (1% Bacto-tryptone, 0.5% Bacto-yeast

extract, 1% NaCl) containing 50 µg/ml of ampicillin, a substance, IPTG, for inducing expression was added to the medium so as to give a final concentration of 1 mM.

Thereafter, the shaking culture was continued for 2 hours at 37°C, thereby producing various human and mouse modified epimorphin polypeptides in Escherichia coli.

Total proteins in the Escherichia coli strain were analyzed by SDS-PAGE. As a result, it was found that all the modified epimorphin fragments were produced in substantially equal amounts.

Example 8:

Investigation as to solubility of the respective modified epimorphin polypeptides

Each of the transformants prepared in Example 7, which separately expressed the human and mouse modified epimorphin polypeptides, was suspended in a Lysis buffer [50 mM Tris-HCl (pH: 8.0), 1 mM EDTA, 100 mM NaCl] to wash it. The thus-washed transformant was subjected to centrifugation to precipitate the bacteria. After the thus-precipitated bacteria were suspended in a Lysis buffer, lysozyme (SIGMA L-6876) was added to the suspension to give a concentration of 1 mg/ml. The mixture was frozen and thawed repeatedly three times to lyse the Escherichia coli strain, followed by its ultrasonic treatment.

Thereafter, the supernatant was removed by centrifugation, and the resultant precipitate was washed 4

times with a 2 M urea/Lysis buffer. The thus-washed precipitate was suspended again in a 8 M urea/Lysis buffer, followed by centrifugation to obtain a supernatant fraction.

5 The thus-obtained supernatant fraction was dialyzed against an excess amount of a PBS buffer and further centrifuged, thereby separating it into a supernatant fraction and a precipitate fraction. The thus-obtained fractions were analyzed by SDS-PAGE to determine the
10 solubility of each modified epimorphin by the proportions of the modified epimorphin existing in the respective fractions.

As a result, as illustrated in FIG. 6, there was shown a tendency for the modified epimorphin polypeptide
15 to become more insoluble as the number of amino acids deleted from the N-terminal side thereof was increased.

Example 9:

Investigation as to activity of the respective modified epimorphin polypeptides

20 As an index to the activity evaluation of the respective modified epimorphin polypeptides, their binding ability to cultured cells was investigated.

The modified epimorphin polypeptides (suspensions in 8 M urea/Lysis buffer) prepared in Example 8 were
25 separately coated on suspension culture dishes and then dried. Thereafter, the thus-coated dishes were washed once with 8 M urea/Lysis buffer and then 5 times with PBS.

Cultured cells CH3/10T1/2 clone 8 (product of Dainippon Pharmaceutical Co., Ltd.) suspended in a D-MEM/F-12 medium (SIGMA D8900) added with 20 mg/ml of BSA (SIGMA-A-7030) were then seeded on the coated dishes.

5 After left over for 1 hour and one day, the dishes were washed 3 times with PBS, and the cells were then lysed with 0.5 N NaOH. The resultant solutions were then recovered and further centrifuged. Thereafter, the amount of DNA in each of the solutions was measured by a
10 spectrophotometer, thereby counting the number of cells attached to the dish to use this value as an index to the activity evaluation of the respective modified epimorphin polypeptides. As illustrated in FIG. 6, the results showed a tendency for the degree of activity to become
15 opposite to the solubility.

Example 10:

Preparation of variant modified epimorphin

(A): cDNA encoding the fragment (2) of mouse epimorphin.

20 (B): Single-stranded DNA obtained by binding a base sequence capable of recognizing a restriction enzyme NdeI to the 5'-terminus of single-stranded DNA composed of 10 to 20 bases from the 5'-terminal side of a sense strand of (A).

25 (C): Single-stranded DNA obtained by binding a base sequence capable of recognizing a restriction enzyme NheI to the 5'-terminus of single-stranded DNA composed of 10

to 20 bases from the 5'-terminal side of an anti-sense strand of (A).

(1) (B) and (C) were produced by a DNA synthesizer.

(2) (A), and (B) and (C) were used as a template
5 and primers, respectively, to obtain double-stranded DNA
by a PCR process. At this time, the PCR was performed
under conditions described in "Technique-a journal of
methods in cell and molecular biology", Vol. 1, No. 1, 11-
15 (August, 1989). By this process, DNAs encoding
10 appropriate variant epimorphin fragments (2), in which
substitution, variation occurred in part of the base
sequence of (A), were obtained.

(3) The DNAs thus obtained were inserted into the
NdeI/NheI site of pET3C, which was obtained by a domain
15 lying between two EcoRV sites thereof, thereby producing a
recombinant vector. This recombinant vector was then
introduced into a host, Escherichia coli strain BL21 in
accordance with the Hanahan process.

(4) This Escherichia coli strain was seeded on an
20 LB plate (1% Bacto-tryptone, 0.5% Bacto-yeast extract, 1%
NaCl, 1.5% Bacto-agar) containing 50 µg/ml of ampicillin
to culture it overnight at 37°C.

(5) In order to identify colonies grown as
transformants having the recombinant vector, an usual PCR
25 process was performed to amplify the DNAs encoding the
variant modified epimorphin fragments intended.
Thereafter, the DNAs were subjected to agarose gel

electrophoresis, thereby confirming its bands.

(6) After the transformants thus obtained were propagated in large amounts by shaking culture at 37°C on liquid LB medium containing 50 µg/ml of ampicillin, substance, IPTG, for inducing expression was added to the medium so as to give a final concentration of 1 mM. Thereafter, the shaking culture was continued for 2 hours, thereby producing various variant modified epimorphin fragments in Escherichia coli.

(7) The Escherichia coli strain of (6) was suspended in Lysis buffer to wash it. The thus-washed strain was subjected to centrifugation to precipitate the bacteria and remove the supernatant. After the thus-precipitated bacteria were recovered and suspended in Lysis buffer, lysozyme was added to the suspension to give a concentration of 1 mg/ml. The mixture was frozen and thawed repeatedly three times to lyse the Escherichia coli strain, followed by its ultrasonic treatment. Thereafter, the supernatant was removed by centrifugation, and the resultant precipitate was washed 4 times with 2 M urea/Lysis buffer. The thus-washed precipitate was suspended again in 8 M urea/Lysis buffer, followed by centrifugation to obtain a supernatant fraction.

Example 11:

Determination of amino acid sequence of variant modified epimorphin

Plasmid DNAs in the Escherichia coli of the colonies

in (5) of Example 10 were prepared by the alkali process ["Laboratory-Manual Gene Engineering" 51-53 (1988), published by Maruzen Co., Ltd.] to analyze the base sequences of the DNAs encoding the variant modified epimorphin fragments by a DNA sequenser. Amino acid sequences encoded by these base sequences were determined to take them as amino acid sequences of their corresponding variant modified epimorphin fragments obtained in Example 10.

10 Example 12:

Evaluation of variant modified epimorphin in cell culture

(1) The variant modified epimorphin fragments (suspensions in 8 M urea/Lysis buffer) prepared in Example 10 were coated on suspension culture dishes to give a coating weight of $10 \mu\text{g}/\text{cm}^2$, and then dried. Thereafter, the thus-coated dishes were washed once with 8 M urea/Lysis buffer and then 5 times with PBS. Cultured cells CH3/10T1/2 clone 8 suspended in a D-MEM/F-12 medium (SIGMA D8900) added with 20 mg/ml of BSA were then seeded on the coated dishes.

(2) After left over for 1 hour, the dishes were washed 3 times with PBS, and the cells were then recovered with 0.5 N NaOH. The value of OD at 260 nm, by which the amount of DNA contained therein was reflected, was determined by a spectrophotometer. The absorbance of an invariant modified epimorphin fragment was 0.33 ± 0.015 . Among the variant modified epimorphin fragments obtained,

six fragments had an absorbance within about this range. This revealed that variants, in which part of their amino acid sequences have been varied, are permissible in the modified epimorphin.

- 5 The varied sites of the variant modified epimorphin fragments, which was produced and evaluated in Examples 10-12, are as shown in Table 2.

Table 2

10	Variant	Varied site (*1)	Original amino acid	Varied amino acid
	a	149th 175th	Ile Ser	Val Pro
	b	175th	Ser	Thr
15	c	115th 127th 130th 131th 139th 154th 166th 177th	Ile Phe Val Met Ile Glu Glu Phe	Val Leu Ala Thr Val Val Asp Leu
20	d	134th 171th 178th 179th 180th	Tyr Ser Ile Ser Asp	Phe Gly Thr Pro Gly
	e	133th 145th 155th 162th 177th	Glu Ser Ile Asp Phe	Val Gly Asn Gly Ser
25	f	122th 115th 131th 155th	Asp Ile Met Ile	Gly Leu Val Phe

(*1) The number counted from the N-terminus of the whole-length mouse epimorphin.

SEQ ID NO : 1 :

(i) SEQUENCE CHARACTERISTICS :

5 (B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Lys Asn Asp Asp

10	1				5					10					15				
	Gly	Asp	Thr	Val	Val	Val	Val	Glu	Lys	Asp	His	Phe	Met	Asp	Asp	Phe			
				20					25					30					
	Phe	His	Gln	Val	Glu	Glu	Ile	Arg	Asn	Ser	Ile	Asp	Lys	Ile	Thr	Gln			
			35					40					45						
15	Tyr	Val	Glu	Glu	Val	Lys	Lys	Asn	His	Ser	Ile	Ile	Leu	Ser	Ala	Pro			
		50					55					60							
	Asn	Pro	Glu	Gly	Lys	Ile	Lys	Glu	Glu	Leu	Glu	Asp	Leu	Asn	Lys	Glu			
	65					70					75					80			
	Ile	Lys	Lys	Thr	Ala	Asn	Lys	Ile	Arg	Ala	Lys	Leu	Lys	Ala	Ile	Glu			
20				85					90					95					
	Gln	Ser	Phe	Asp	Gln	Asp	Glu	Ser	Gly	Asn	Arg	Thr	Ser	Val	Asp	Leu			
			100						105					110					
	Arg	Ile	Arg	Arg	Thr	Gln	His	Ser	Val	Leu	Ser	Arg	Lys	Phe	Val	Glu			
		115					120					125							
25	Ala	Met	Ala	Glu	Tyr	Asn	Glu	Ala	Gln	Thr	Leu	Phe	Arg	Glu	Arg	Ser			
		130					135					140							
	Lys	Gly	Arg	Ile	Gln	Arg	Gln	Leu	Glu	Ile	Thr	Gly	Arg	Thr	Thr	Thr			
	145				150					155						160			

SEQUENCE TABLE

SEQ ID NO : 1 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 288 amino acids

5 (B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 1 :

	Met	Arg	Asp	Arg	Leu	Pro	Asp	Leu	Thr	Ala	Cys	Arg	Lys	Asn	Asp	Asp
10	1				5					10					15	
	Gly	Asp	Thr	Val	Val	Val	Val	Glu	Lys	Asp	His	Phe	Met	Asp	Asp	Phe
					20					25					30	
	Phe	His	Gln	Val	Glu	Glu	Ile	Arg	Asn	Ser	Ile	Asp	Lys	Ile	Thr	Gln
					35					40					45	
15	Tyr	Val	Glu	Glu	Val	Lys	Lys	Asn	His	Ser	Ile	Ile	Leu	Ser	Ala	Pro
					50					55					60	
	Asn	Pro	Glu	Gly	Lys	Ile	Lys	Glu	Glu	Leu	Glu	Asp	Leu	Asn	Lys	Glu
					65					70					75	80
	Ile	Lys	Lys	Thr	Ala	Asn	Lys	Ile	Arg	Ala	Lys	Leu	Lys	Ala	Ile	Glu
20					85					90					95	
	Gln	Ser	Phe	Asp	Gln	Asp	Glu	Ser	Gly	Asn	Arg	Thr	Ser	Val	Asp	Leu
					100					105					110	
	Arg	Ile	Arg	Arg	Thr	Gln	His	Ser	Val	Leu	Ser	Arg	Lys	Phe	Val	Glu
					115					120					125	
25	Ala	Met	Ala	Glu	Tyr	Asn	Glu	Ala	Gln	Thr	Leu	Phe	Arg	Glu	Arg	Ser
					130					135					140	
	Lys	Gly	Arg	Ile	Gln	Arg	Gln	Leu	Glu	Ile	Thr	Gly	Arg	Thr	Thr	Thr
					145					150					155	160

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SEQ ID NO : 2 :

20 (A) LENGTH : 287 amino acids
 (B) TYPE : amino acid
 (D) TOPOLOGY : linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Lys Asn Asp Asp
1 5 10 15

Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
 20 25 30
 Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
 35 40 45
 5 Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
 50 55 60
 Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
 65 70 75 80
 Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
 10 85 90 95
 Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
 100 105 110
 Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
 115 120 125
 15 Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
 130 135 140
 Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
 145 150 155 160
 Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile Phe
 20 165 170 175
 Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu Asn
 180 185 190
 Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser Ile
 195 200 205
 25 Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu Thr
 210 215 220
 Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met Asn Ala Thr
 225 230 235 240

Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
 20 25 30
 Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
 35 40 45
 5 Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
 50 55 60
 Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
 65 70 75 80
 Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
 10 85 90 95
 Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
 100 105 110
 Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
 115 120 125
 15 Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
 130 135 140
 Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
 145 150 155 160
 Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile Phe
 20 165 170 175
 Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu Asn
 180 185 190
 Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser Ile
 195 200 205
 25 Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu Thr
 210 215 220
 Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met Asn Ala Thr
 225 230 235 240

Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys Thr
 245 250 255
 Gln Ser Lys Ala Arg Arg Lys Leu Met Phe Ile Ile Ile Cys Val Ile
 260 265 270
 5 Val Leu Leu Val Ile Leu Gly Ile Ile Leu Ala Thr Thr Leu Ser
 275 280 285

SEQ ID NO : 3 :

(i) SEQUENCE CHARACTERISTICS :

- 10 (A) LENGTH : 277 amino acids
 (B) TYPE : amino acid
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 3 :

15 Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Lys Asn Asp Asp
 1 5 10 15
 Gly Asp Thr Val Val Val Val Glu Lys Asp His Phe Met Asp Asp Phe
 20 25 30
 Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp Lys Ile Thr Gln
 20 35 40 45
 Tyr Val Glu Glu Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala Pro
 50 55 60
 Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys Glu
 65 70 75 80
 25 Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys Ala Ile Glu
 85 90 95
 Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser Val Asp Leu
 100 105 110

Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val Glu
 115 120 125
 Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg Glu Arg Ser
 130 135 140
 5 Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr Thr
 145 150 155 160
 Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile Phe
 165 170 175
 Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu Asn
 10 180 185 190
 Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser Ile
 195 200 205
 Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu Thr
 210 215 220
 15 Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met Asn Ala Thr
 225 230 235 240
 Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys Tyr
 245 250 255
 Gln Ser Lys Ala Arg Arg Gln Gln His Cys His Ser Asn His Ile Pro
 20 260 265 270
 Arg Ala Ile Tyr Pro
 275

SEQ ID NO : 4 :

- 25 (i) SEQUENCE CHARACTERISTICS :
- (A) LENGTH : 289 amino acids
 - (B) TYPE : amino acid
 - (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 4 :

	Met	Arg	Asp	Arg	Leu	Pro	Asp	Leu	Thr	Ala	Cys	Arg	Thr	Asn	Asp	Asp
	1				5					10					15	
5	Gly	Asp	Thr	Ala	Val	Val	Ile	Val	Glu	Lys	Asp	His	Phe	Met	Asp	Gly
				20					25					30		
	Phe	Phe	His	Gln	Val	Glu	Glu	Ile	Arg	Ser	Ser	Ile	Ala	Arg	Ile	Ala
			35					40					45			
	Gln	His	Val	Glu	Asp	Val	Lys	Lys	Asn	His	Ser	Ile	Ile	Leu	Ser	Ala
10		50					55					60				
	Pro	Asn	Pro	Glu	Gly	Lys	Ile	Lys	Glu	Glu	Leu	Glu	Asp	Leu	Asn	Lys
		65				70				75				80		
	Glu	Ile	Lys	Lys	Thr	Ala	Asn	Arg	Ile	Arg	Gly	Lys	Leu	Lys	Ser	Ile
				85				90					95			
15	Glu	Gln	Ser	Cys	Asp	Gln	Asp	Glu	Asn	Gly	Asn	Arg	Thr	Ser	Val	Asp
				100				105					110			
	Leu	Arg	Ile	Arg	Arg	Thr	Gln	His	Ser	Val	Leu	Ser	Arg	Lys	Phe	Val
			115				120					125				
	Asp	Val	Met	Thr	Glu	Tyr	Asn	Glu	Ala	Gln	Ile	Leu	Phe	Arg	Glu	Arg
20		130					135					140				
	Ser	Lys	Gly	Arg	Ile	Gln	Arg	Gln	Leu	Glu	Ile	Thr	Gly	Arg	Thr	Thr
		145				150				155				160		
	Thr	Asp	Asp	Glu	Leu	Glu	Glu	Met	Leu	Glu	Ser	Gly	Lys	Pro	Ser	Ile
				165				170					175			
25	Phe	Ile	Ser	Asp	Ile	Ile	Ser	Asp	Ser	Gln	Ile	Thr	Arg	Gln	Ala	Leu
				180				185					190			
	Asn	Glu	Ile	Glu	Ser	Arg	His	Lys	Asp	Ile	Met	Lys	Leu	Glu	Thr	Ser
				195				200					205			

Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu
 210 215 220
 Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val Val Asn Ser
 225 230 235 240
 5 Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys
 245 250 255
 Tyr Gln Ser Lys Ala Arg Arg Lys Lys Trp Ile Ile Ala Ala Val Ala
 260 265 270
 Val Ala Val Ile Ala Val Leu Ala Leu Ile Ile Gly Leu Ser Val Gly
 10 275 280 285
 Lys

SEQ ID NO : 5 :

(i) SEQUENCE CHARACTERISTICS :

- 15 (A) LENGTH : 288 amino acids
 (B) TYPE : amino acid
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 5 :

20 Met Arg Asp Arg Leu Pro Asp Leu Thr Ala Cys Arg Thr Asn Asp Asp
 1 5 10 15
 Gly Asp Thr Ala Val Val Ile Val Glu Lys Asp His Phe Met Asp Gly
 20 25 30
 Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala Arg Ile Ala
 25 35 40 45
 Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile Leu Ser Ala
 50 55 60

Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp Leu Asn Lys
 65 70 75 80
 Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu Lys Ser Ile
 85 90 95
 5 Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr Ser Val Asp
 100 105 110
 Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys Phe Val
 115 120 125
 Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe Arg Glu Arg
 10 130 135 140
 Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg Thr Thr
 145 150 155 160
 Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile
 165 170 175
 15 Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu
 180 185 190
 Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser
 195 200 205
 Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu
 20 210 215 220
 Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val Val Asn Ser
 225 230 235 240
 Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys
 245 250 255
 25 Tyr Gln Ser Lys Ala Arg Arg Lys Val Met Phe Val Leu Ile Cys Val
 260 265 270
 Val Thr Leu Leu Val Ile Leu Gly Ile Ile Leu Ala Thr Ala Leu Ser
 275 280 285

SEQ ID NO : 6 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 279 amino acids

(B) TYPE : amino acid

5 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 6 :

	Met	Arg	Asp	Arg	Leu	Pro	Asp	Leu	Thr	Ala	Cys	Arg	Thr	Asn	Asp	Asp
	1				5					10					15	
10	Gly	Asp	Thr	Ala	Val	Val	Ile	Val	Glu	Lys	Asp	His	Phe	Met	Asp	Gly
					20					25					30	
	Phe	Phe	His	Gln	Val	Glu	Glu	Ile	Arg	Ser	Ser	Ile	Ala	Arg	Ile	Ala
					35					40					45	
	Gln	His	Val	Glu	Asp	Val	Lys	Lys	Asn	His	Ser	Ile	Ile	Leu	Ser	Ala
15		50						55						60		
	Pro	Asn	Pro	Glu	Gly	Lys	Ile	Lys	Glu	Glu	Leu	Glu	Asp	Leu	Asn	Lys
		65					70				75				80	
	Glu	Ile	Lys	Lys	Thr	Ala	Asn	Arg	Ile	Arg	Gly	Lys	Leu	Lys	Ser	Ile
					85					90					95	
20	Glu	Gln	Ser	Cys	Asp	Gln	Asp	Glu	Asn	Gly	Asn	Arg	Thr	Ser	Val	Asp
					100					105					110	
	Leu	Arg	Ile	Arg	Arg	Thr	Gln	His	Ser	Val	Leu	Ser	Arg	Lys	Phe	Val
					115					120					125	
	Asp	Val	Met	Thr	Glu	Tyr	Asn	Glu	Ala	Gln	Ile	Leu	Phe	Arg	Glu	Arg
25		130						135					140			
	Ser	Lys	Gly	Arg	Ile	Gln	Arg	Gln	Leu	Glu	Ile	Thr	Gly	Arg	Thr	Thr
					145				150				155			160

Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro Ser Ile
 165 170 175
 Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln Ala Leu
 180 185 190
 5 Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu Thr Ser
 195 200 205
 Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe Val Glu
 210 215 220
 Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val Val Asn Ser
 10 225 230 235 240
 Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala Ile Lys
 245 250 255
 Tyr Gln Ser Lys Ala Arg Arg Gln Gln His Cys His Ser Asn Arg Thr
 260 265 270
 15 Pro Arg Ala Leu Cys Pro Arg
 275

SEQ ID NO : 7 :

(i) SEQUENCE CHARACTERISTICS :

- 20 (A) LENGTH : 237 base pairs
 (B) TYPE : nucleic acid
 (C) STRANDEDNESS : double
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

25 (vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 7 :

ATG GAT GAT TTC TTC CAT CAG GTG GAG GAG AAT AGA AAC AGT ATT GAT 16

AAA ATA ACT CAA TAT GTT GAA GAA GTA AAG AAA AAC CAC AGC ATC ATT 32
 CTT TCT GCA CCA AAC CCG GAA GGA AAA ATA AAA GAA GAG CTT GAA GAT 48
 CTG AAC AAA GAA ATC AAG AAA ACT GCG AAT AAA ATT CGA GCC AAG TTA 64
 AAG GCT ATT GAA CAA AGT TTT GAT CAG GAT GAG AGT GGG AAC CGG ACT 80
 5 TCA GTG GAT CTT CGG ATA CGA AGA ACC CAG CAT TCG GTG CTG TCT CGG 96
 AAG TTT GTG GAA GCC ATG GCG GAG TAC AAT GAG GCA CAG ACT CTG TTT 112
 CGG GAG CGG AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATA ACT GGG 128
 AGA ACC ACC ACA GAC GAC GAG CTA GAA GAG ATG CTG GAG AGC GGG AAG 144
 CCA TCC ATC TTC ACT TCC GAC ATT ATA TCA GAT TCA CAA ATT ACT AGA 160
 10 CAA GCT CTC AAT GAA ATC GAG TCA CGT CAC AAG GAC ATC ATG AAG CTG 176
 GAG ACC AGC ATC CGA GAG TTG CAT GAG ATG TTC ATG GAC ATG GCT ATG 192
 TTT GTG GAG ACT CAG GGT GAA ATG ATC AAC AAC ATA GAA AGA AAT GTT 208
 ATG AAT GCC ACA GAC TAT GTA GAA CAC GCT AAA GAA GAA ACA AAA AAA 224
 GCT ATC AAA TAT CAG AGC AAG GCA AGA AGG AAA AAG TGA 237

15

SEQ ID NO : 8 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 188 base pairs

(B) TYPE : nucleic acid

20 (C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

25 (xi) SEQUENCE DESCRIPTION : SEQ ID NO : 8 :

AAC AAA GAA ATC AAG AAA ACT GCG AAT AAA ATT CGA GCC AAG TTA AAG 16
 GCT ATT GAA CAA AGT TTT GAT CAG GAT GAG AGT GGG AAC CGG ACT TCA 32
 GTG GAT CTT CGG ATA CGA AGA ACC CAG CAT TCG GTG CTG TCT CGG AAG 48

TTT GTG GAA GCC ATG GCG GAG TAC AAT GAG GCA CAG ACT CTG TTT CGG 64
 GAG CGG AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATA ACT GGG AGA 80
 ACC ACC ACA GAC GAC GAG CTA GAA GAG ATG CTG GAG AGC GGG AAG CCA 96
 TCC ATC TTC ACT TCC GAC ATT ATA TCA GAT TCA CAA ATT ACT AGA CAA 112
 5 GCT CTC AAT GAA ATC GAG TCA CGT CAC AAG GAC ATC ATG AAG CTG GAG 128
 ACC AGC ATC CGA GAG TTG CAT GAG ATG TTC ATG GAC ATG GCT ATG TTT 144
 GTG GAG ACT CAG GGT GAA ATG ATC AAC AAC ATA GAA AGA AAT GTT ATG 160
 AAT GCC ACA GAC TAT GTA GAA CAC GCT AAA GAA GAA ACA AAA AAA GCT 176
 ATC AAA TAT CAG AGC AAG GCA AGA AGG AAA AAG TGA 188

10

SEQ ID NO : 9 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 162 base pairs

(B) TYPE : nucleic acid

15 (C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

20 (xi) SEQUENCE DESCRIPTION : SEQ ID NO : 9 :

AGT GGG AAC CGG ACT TCA GTG GAT CTT CGG ATA CGA AGA ACC CAG CAT 16
 TCG GTG CTG TCT CGG AAG TTT GTG GAA GCC ATG GCG GAG TAC AAT GAG 32
 GCA CAG ACT CTG TTT CGG GAG CGG AGC AAA GGC CGC ATC CAG CGC CAG 48
 CTG GAG ATA ACT GGG AGA ACC ACC ACA GAC GAC GAG CTA GAA GAG ATG 64
 25 CTG GAG AGC GGG AAG CCA TCC ATC TTC ACT TCC GAC ATT ATA TCA GAT 80
 TCA CAA ATT ACT AGA CAA GCT CTC AAT GAA ATC GAG TCA CGT CAC AAG 96
 GAC ATC ATG AAG CTG GAG ACC AGC ATC CGA GAG TTG CAT GAG ATG TTC 112
 ATG GAC ATG GCT ATG TTT GTG GAG ACT CAG GGT GAA ATG ATC AAC AAC 128

ATA GAA AGA AAT GTT ATG AAT GCC ACA GAC TAT GTA GAA CAC GCT AAA 144
 GAA GAA ACA AAA AAA GCT ATC AAA TAT CAG AGC AAG GCA AGA AGG AAA 160
 AAG TGA 162

5 SEQ ID NO : 10 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 265 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

10 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Homo sapiens

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 10 :

15 ATG CGG GAC CGG CTG CCA GAC CTG ACG GCG TGT AGG AAG AAT GAT GAT 16
 GGA GAC ACA GTT GTT GTG GTT GAG AAA GAT CAT TTC ATG GAT GAT TTC 32
 TTC CAT CAG GTG GAG GAG ATT AGA AAC AGT ATT GAT AAA ATA ACT CAA 48
 TAT GTT GAA GAA GTA AAG AAA AAC CAC AGC ATC ATT CTT TCT GCA CCA 64
 AAC CCG GAA GGA AAA ATA AAA GAA GAG CTT GAA GAT CTG AAC AAA GAA 80
 20 ATC AAG AAA ACT GCG AAT AAA ATT CGA GCC AAG TTA AAG GCT ATT GAA 96
 CAA AGT TTT GAT CAG GAT GAG AGT GGG AAC CGG ACT TCA GTG GAT CTT 112
 CGG ATA CGA AGA ACC CAG CAT TCG GTG CTG TCT CGG AAG TTT GTG GAA 128
 GCC ATG GCG GAG TAC AAT GAG GCA CAG ACT CTG TTT CGG GAG CGG AGC 144
 AAA GGC CGC ATC CAG CGC CAG CTG GAG ATA ACT GGG AGA ACC ACC ACA 160
 25 GAC GAC GAG CTA GAA GAG ATG CTG GAG AGC GGG AAG CCA TCC ATC TTC 176
 ACT TCC GAC ATT ATA TCA GAT TCA CAA ATT ACT AGA CAA GCT CTC AAT 192
 GAA ATC GAG TCA CGT CAC AAG GAC ATC ATG AAG CTG GAG ACC AGC ATC 208
 CGA GAG TTG CAT GAG ATG TTC ATG GAC ATG GCT ATG TTT GTG GAG ACT 224

CAG GGT GAA ATG ATC AAC AAC ATA GAA AGA AAT GTT ATG AAT GCC ACA 240
 GAC TAT GTA GAA CAC GCT AAA GAA GAA ACA AAA AAA GCT ATC AAA TAT 256
 CAG AGC AAG GCA AGA AGG AAA AAG TGA 265

5 SEQ ID NO : 11 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 237 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

10 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 11 :

15 ATG GAC GGT TTC TTC CAT CAG GTA GAG GAG ATT CGA AGC AGC ATA GCC 16
 AGG ATT GCT CAG CAT GTA GAA GAC GTG AAG AAG AAC CAC AGC ATC ATC 32
 CTG TCT GCT CCA AAC CCA GAA GGA AAA ATA AAA GAA GAG CTG GAG GAC 48
 CTG AAC AAA GAG ATC AAG AAA ACT GCT AAC AGG ATC CGG GGC AAG CTG 64
 AAG TCT ATT GAG CAG AGC TGT GAT CAG GAC GAG AAT GGG AAC CGA ACT 80
 20 TCA GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC TCG GTG CTG TCA CGG 96
 AAG TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA GCG CAG ATC CTG TTC 112
 CGG GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATC ACT GGG 128
 AGG ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG CTG GAG AGC GGG AAG 144
 CCG TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT TCA CAA ATC ACT AGG 160
 25 CAA GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA GAC ATC ATG AAG CTG 176
 GAG ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC ATG GAT ATG GCC ATG 192
 TTT GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC ATC GAG AGA AAT GTG 208
 GTG AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG GAA GAG ACG AAG AAA 224

GCC ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA AAG TGA

237

SEQ ID NO : 12 :

(i) SEQUENCE CHARACTERISTICS :

- 5 (A) LENGTH : 188 base pairs
(B) TYPE : nucleic acid
(C) STRANDEDNESS : double
(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

10 (vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 12 :

AAC AAA GAG ATC AAG AAA ACT GCT AAC AGG ATC CGG GGC AAG CTG AAG 16
TCT ATT GAG CAG AGC TGT GAT CAG GAC GAG AAT GGG AAC CGA ACT TCA 32
15 GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC TCG GTG CTG TCA CGG AAG 48
TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA GCG CAG ATC CTG TTC CGG 64
GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATC ACT GGG AGG 80
ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG CTG GAG AGC GGG AAG CCG 96
TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT TCA CAA ATC ACT AGG CAA 112
20 GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA GAC ATC ATG AAG CTG GAG 128
ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC ATG GAT ATG GCC ATG TTT 144
GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC ATC GAG AGA AAT GTG GTG 160
AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG GAA GAG ACG AAG AAA GCC 176
ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA AAG TGA 188

25

SEQ ID NO : 13 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 162 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

5 (vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 13 :

	AAT GGG AAC CGA ACT TCA GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC	16
	TCG GTG CTG TCA CGG AAG TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA	32
10	GCG CAG ATC CTG TTC CGG GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG	48
	CTG GAG ATC ACT GGG AGG ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG	64
	CTG GAG AGC GGG AAG CCG TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT	80
	TCA CAA ATC ACT AGG CAA GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA	96
	GAC ATC ATG AAG CTG GAG ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC	112
15	ATG GAT ATG GCC ATG TTT GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC	128
	ATC GAG AGA AAT GTG GTG AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG	144
	GAA GAG ACG AAG AAA GCC ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA	160
	AAG TGA	162

20 SEQ ID NO : 14 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 266 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

25 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

5 (vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 13 :

	AAT GGG AAC CGA ACT TCA GTG GAT CTG CGG ATA CGA AGG ACC CAG CAC	16
	TCG GTG CTG TCA CGG AAG TTT GTG GAC GTC ATG ACA GAA TAC AAT GAA	32
10	GCG CAG ATC CTG TTC CGG GAG CGA AGC AAA GGC CGC ATC CAG CGC CAG	48
	CTG GAG ATC ACT GGG AGG ACC ACC ACT GAC GAC GAG CTG GAA GAG ATG	64
	CTG GAG AGC GGG AAG CCG TCC ATC TTC ATC TCG GAT ATT ATA TCA GAT	80
	TCA CAA ATC ACT AGG CAA GCT CTC AAT GAG ATC GAG TCC CGC CAC AAA	96
	GAC ATC ATG AAG CTG GAG ACC AGC ATC CGA GAG CTG CAC GAG ATG TTC	112
15	ATG GAT ATG GCC ATG TTT GTC GAG ACT CAG GGT GAA ATG GTC AAC AAC	128
	ATC GAG AGA AAT GTG GTG AAC TCT GTA GAT TAC GTG GAA CAT GCC AAG	144
	GAA GAG ACG AAG AAA GCC ATC AAA TAC CAG AGC AAG GCC AGG CGG AAA	160
	AAG TGA	162

20 SEQ ID NO : 14 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 266 base pairs

(B) TYPE : nucleic acid

(C) STRANDEDNESS : double

25 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : cDNA

(vi) ORIGINAL SOURCE :

(A) ORGANISM : Mus

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 14 :

	ATG CGG GAC CGG CTG CCC GAC CTC ACG GCG TGT AGG ACA AAC GAC GAT	16
	GGA GAC ACT GCT GTC GTC ATT GTG GAG AAG GAT CAT TTC ATG GAC GGT	32
	TTC TTC CAT CAG GTA GAG GAG ATT CGA AGC AGC ATA GCC AGG ATT GCT	48
5	CAG CAT GTA GAA GAC GTG AAG AAG AAC CAC AGC ATC ATC CTG TCT GCT	64
	CCA AAC CCA GAA GGA AAA ATA AAA GAA GAG CTG GAG GAC CTG GAC AAA	80
	GAG ATC AAG AAA ACT GCT AAC AGG ATC CGG GGC AAG CTG AAG TCT ATT	96
	GAG CAG AGC TGT GAT CAG GAC GAG AAT GGG AAC CGA ACT TCA GTG GAT	112
	CTG CGG ATA CGA AGG ACC CAG CAC TCG GTG CTG TCA CGG AAG TTT GTG	128
10	GAC GTC ATG ACA GAA TAC AAT GAA GCG CAG ATC CTG TTC CGG GAG CGA	144
	AGC AAA GGC CGC ATC CAG CGC CAG CTG GAG ATC ACT GGG AGG ACC ACC	160
	ACT GAC GAC GAG CTG GAA GAG ATG CTG GAG AGC GGG AAG CCG TCC ATC	176
	TTC ATC TCG GAT ATT ATA TCA GAT TCA CAA ATC ACT AGG CAA GCT CTC	192
	AAT GAG ATC GAG TCC CGC CAC AAA GAC ATC ATG AAG CTG GAG ACC AGC	208
15	ATC CGA GAG CTG CAC GAG ATG TTC ATG GAT ATG GCC ATG TTT GTC GAG	224
	ACT CAG GGT GAA ATG GTC AAC AAC ATC GAG AGA AAT GTG GTG AAC TCT	240
	GTA GAT TAC GTG GAA CAT GCC AAG GAA GAG ACG AAG AAA GCC ATC AAA	256
	TAC CAG AGC AAG GCC AGG CGG AAA AAG TGA	266

20 SEQ ID NO : 15 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 236 amino acids

(B) TYPE : amino acid

(D) TOPOLOGY : linear

25 (ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 15 :

Met Asp Asp Phe Phe His Gln Val Glu Glu Ile Arg Asn Ser Ile Asp

1

5

10

15

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	Lys	Ile	Thr	Gln	Tyr	Val	Glu	Glu	Val	Lys	Lys	Asn	His	Ser	Ile	Ile	
	20					25					30						
	Leu	Ser	Ala	Pro	Asn	Pro	Glu	Gly	Lys	Ile	Lys	Glu	Glu	Leu	Glu	Asp	
	35					40					45						
5	Leu	Asn	Lys	Glu	Ile	Lys	Lys	Thr	Ala	Asn	Lys	Ile	Arg	Ala	Lys	Leu	
	50					55					60						
	Lys	Ala	Ile	Glu	Gln	Ser	Phe	Asp	Gln	Asp	Glu	Ser	Gly	Asn	Arg	Thr	
	65					70					75					80	
	Ser	Val	Asp	Leu	Arg	Ile	Arg	Arg	Thr	Gln	His	Ser	Val	Leu	Ser	Arg	
10	85					90					95						
	Lys	Phe	Val	Glu	Ala	Met	Ala	Glu	Tyr	Asn	Glu	Ala	Gln	Thr	Leu	Phe	
	100					105					110						
	Arg	Glu	Arg	Ser	Lys	Gly	Arg	Ile	Gln	Arg	Gln	Leu	Glu	Ile	Thr	Gly	
	115					120					125						
15	Arg	Thr	Thr	Thr	Asp	Asp	Glu	Leu	Glu	Glu	Met	Leu	Glu	Ser	Gly	Lys	
	130					135					140						
	Pro	Ser	Ile	Phe	Thr	Ser	Asp	Ile	Ile	Ser	Asp	Ser	Gln	Ile	Thr	Arg	
	145					150					155					160	
	Gln	Ala	Leu	Asn	Glu	Ile	Glu	Ser	Arg	His	Lys	Asp	Ile	Met	Lys	Leu	
20	165					170					175						
	Glu	Thr	Ser	Ile	Arg	Glu	Leu	His	Glu	Met	Phe	Met	Asp	Met	Ala	Met	
	180					185					190						
	Phe	Val	Glu	Thr	Gln	Gly	Glu	Met	Ile	Asn	Asn	Ile	Glu	Arg	Asn	Val	
	195					200					205						
25	Met	Asn	Ala	Thr	Asp	Tyr	Val	Glu	His	Ala	Lys	Glu	Glu	Thr	Lys	Lys	
	210					215					220						
	Ala	Ile	Lys	Thr	Gln	Ser	Lys	Ala	Arg	Arg	Lys	Lys					
	225					230					235						

(i) SEQUENCE CHARACTERISTICS :

(B) TYPE : amino acid

5 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Asn Lys Glu Ile Lys Lys Thr Ala Asn Lys Ile Arg Ala Lys Leu Lys

1 5 10 15

10 Ala Ile Glu Gln Ser Phe Asp Gln Asp Glu Ser Gly Asn Arg Thr Ser

20 25 30

Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg Lys

35 40 45

Phe Val Glu Ala Met Ala Glu Tyr Asn Glu Ala Gln Thr Leu Phe Arg

15 50 55 60

Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly Arg

65 70 75 80

Thr Thr Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys Pro

85 90 95

20 Ser Ile Phe Thr Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg Gln

100 105 110

Ala Leu Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu Glu

115

Thr Ser Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met Phe

25 130 135 140

Val Glu Thr Gln Gly Glu Met Ile Asn Asn Ile Glu Arg Asn Val Met

145 150 155 160

Asn Ala Thr Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys Ala

165

170

175

Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys Lys

180

185

5

SEQ ID NO : 17 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 161 amino acids

(B) TYPE : amino acid

10

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 17 :

Ser Gly Asn Arg Thr Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His

1

5

10

15

15

Ser Val Leu Ser Arg Lys Phe Val Glu Ala Met Ala Glu Tyr Asn Glu

20

25

30

Ala Gln Thr Leu Phe Arg Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln

35

40

45

Leu Glu Ile Thr Gly Arg Thr Thr Thr Asp Asp Glu Leu Glu Glu Met

20

50

55

60

Leu Glu Ser Gly Lys Pro Ser Ile Phe Thr Ser Asp Ile Ile Ser Asp

65

70

75

80

Ser Gln Ile Thr Arg Gln Ala Leu Asn Glu Ile Glu Ser Arg His Lys

85

90

95

25

Asp Ile Met Lys Leu Glu Thr Ser Ile Arg Glu Leu His Glu Met Phe

100

105

110

Met Asp Met Ala Met Phe Val Glu Thr Gln Gly Glu Met Ile Asn Asn

115

120

125

Ile Glu Arg Asn Val Met Asn Ala Thr Asp Tyr Val Glu His Ala Lys

130

135

140

Glu Glu Thr Lys Lys Ala Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys

145

150

155

160

5 Lys

SEQ ID NO : 18 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 236 amino acids

10 (B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 18 :

Met Asp Gly Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala

15

1

5

10

15

Arg Ile Ala Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile

20

25

30

Leu Ser Ala Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp

35

40

45

20 Leu Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu

50

55

60

Lys Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr

65

70

75

80

Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg

25

85

90

95

Lys Phe Val Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe

100

105

110

Ile Glu Arg Asn Val Met Asn Ala Thr Asp Tyr Val Glu His Ala Lys

130

135

140

Glu Glu Thr Lys Lys Ala Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys

145

150

155

160

5 Lys

SEQ ID NO : 18 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 236 amino acids

10 (B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 18 :

Met Asp Gly Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala

15

1

5

10

15

Arg Ile Ala Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile

20

25

30

Leu Ser Ala Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp

35

40

45

20

Leu Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu

50

55

60

Lys Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr

65

70

75

80

Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg

25

85

90

95

Lys Phe Val Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe

100

105

110

Ile Glu Arg Asn Val Met Asn Ala Thr Asp Tyr Val Glu His Ala Lys
 130 135 140
 Glu Glu Thr Lys Lys Ala Ile Lys Thr Gln Ser Lys Ala Arg Arg Lys
 145 150 155 160
 5 Lys

SEQ ID NO : 18 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 236 amino acids
 10 (B) TYPE : amino acid
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 18 :

Met Asp Gly Phe Phe His Gln Val Glu Glu Ile Arg Ser Ser Ile Ala
 15 1 5 10 15
 Arg Ile Ala Gln His Val Glu Asp Val Lys Lys Asn His Ser Ile Ile
 20 20 25 30
 Leu Ser Ala Pro Asn Pro Glu Gly Lys Ile Lys Glu Glu Leu Glu Asp
 35 40 45
 20 Leu Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu
 50 55 60
 Lys Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr
 65 70 75 80
 Ser Val Asp Leu Arg Ile Arg Arg Thr Gln His Ser Val Leu Ser Arg
 25 85 90 95
 Lys Phe Val Asp Val Met Thr Glu Tyr Asn Glu Ala Gln Ile Leu Phe
 100 105 110

Arg Glu Arg Ser Lys Gly Arg Ile Gln Arg Gln Leu Glu Ile Thr Gly
 115 120 125
 Arg Thr Thr Thr Asp Asp Glu Leu Glu Glu Met Leu Glu Ser Gly Lys
 130 135 140
 5 Pro Ser Ile Phe Ile Ser Asp Ile Ile Ser Asp Ser Gln Ile Thr Arg
 145 150 155 160
 Gln Ala Leu Asn Glu Ile Glu Ser Arg His Lys Asp Ile Met Lys Leu
 165 170 175
 Glu Thr Ser Ile Arg Glu Leu His Glu Met Phe Met Asp Met Ala Met
 10 180 185 190
 Phe Val Glu Thr Gln Gly Glu Met Val Asn Asn Ile Glu Arg Asn Val
 195 200 205
 Val Asn Ser Val Asp Tyr Val Glu His Ala Lys Glu Glu Thr Lys Lys
 210 215 220
 15 Ala Ile Lys Tyr Gln Ser Lys Ala Arg Arg Lys Lys
 225 230 235

SEQ ID NO : 19 :

(i) SEQUENCE CHARACTERISTICS :

- 20 (A) LENGTH : 187 amino acids
 (B) TYPE : amino acid
 (D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 19 :

25 Asn Lys Glu Ile Lys Lys Thr Ala Asn Arg Ile Arg Gly Lys Leu Lys
 1 5 10 15
 Ser Ile Glu Gln Ser Cys Asp Gln Asp Glu Asn Gly Asn Arg Thr Ser
 20 25 30

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	Val	Asp	Leu	Arg	Ile	Arg	Arg	Thr	Gln	His	Ser	Val	Leu	Ser	Arg	Lys
			35					40					45			
	Phe	Val	Asp	Val	Met	Thr	Glu	Tyr	Asn	Glu	Ala	Gln	Ile	Leu	Phe	Arg
			50				55					60				
5	Glu	Arg	Ser	Lys	Gly	Arg	Ile	Gln	Arg	Gln	Leu	Glu	Ile	Thr	Gly	Arg
	65					70					75				80	
	Thr	Thr	Thr	Asp	Asp	Glu	Leu	Glu	Glu	Met	Leu	Glu	Ser	Gly	Lys	Pro
						85				90					95	
	Ser	Ile	Phe	Ile	Ser	Asp	Ile	Ile	Ser	Asp	Ser	Gln	Ile	Thr	Arg	Gln
10				100					105					110		
	Ala	Leu	Asn	Glu	Ile	Glu	Ser	Arg	His	Lys	Asp	Ile	Met	Lys	Leu	Glu
				115					120					125		
	Thr	Ser	Ile	Arg	Glu	Leu	His	Glu	Met	Phe	Met	Asp	Met	Ala	Met	Phe
				130					135					140		
15	Val	Glu	Thr	Gln	Gly	Glu	Met	Val	Asn	Asn	Ile	Glu	Arg	Asn	Val	Val
	145					150					155				160	
	Asn	Ser	Val	Asp	Tyr	Val	Glu	His	Ala	Lys	Glu	Glu	Thr	Lys	Lys	Ala
						165					170				175	
	Ile	Lys	Tyr	Gln	Ser	Lys	Ala	Arg	Arg	Lys	Lys					
20						180					185					

SEQ ID NO : 20 :

(i) SEQUENCE CHARACTERISTICS :

(A) LENGTH : 161 amino acids

25 (B) TYPE : amino acid

(D) TOPOLOGY : linear

(ii) MOLECULE TYPE : peptide

(xi) SEQUENCE DESCRIPTION : SEQ ID NO : 20 :

2152210

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	Asn	Gly	Asn	Arg	Thr	Ser	Val	Asp	Leu	Arg	Ile	Arg	Arg	Thr	Gln	His
	1				5					10					15	
	Ser	Val	Leu	Ser	Arg	Lys	Phe	Val	Asp	Val	Met	Thr	Glu	Tyr	Asn	Glu
					20					25					30	
5	Ala	Gln	Ile	Leu	Phe	Arg	Glu	Arg	Ser	Lys	Gly	Arg	Ile	Gln	Arg	Gln
					35					40					45	
	Leu	Glu	Ile	Thr	Gly	Arg	Thr	Thr	Thr	Asp	Asp	Glu	Leu	Glu	Glu	Met
					50					55					60	
	Leu	Glu	Ser	Gly	Lys	Pro	Ser	Ile	Phe	Ile	Ser	Asp	Ile	Ile	Ser	Asp
10		65				75					75				80	
	Ser	Gln	Ile	Thr	Arg	Gln	Ala	Leu	Asn	Glu	Ile	Glu	Ser	Arg	His	Lys
					85						90				95	
	Asp	Ile	Met	Lys	Leu	Glu	Thr	Ser	Ile	Arg	Glu	Leu	His	Glu	Met	Phe
					100					105					110	
15	Met	Asp	Met	Ala	Met	Phe	Val	Glu	Thr	Gln	Gly	Glu	Met	Val	Asn	Asn
					115					125					125	
	Ile	Glu	Arg	Asn	Val	Val	Asn	Ser	Val	Asp	Tyr	Val	Glu	His	Ala	Lys
					130					135					140	
	Glu	Glu	Thr	Lys	Lys	Ala	Ile	Lys	Tyr	Gln	Ser	Lys	Ala	Arg	Arg	Lys
20		145				150					155				160	
	Lys															

Claimed:

1 1. A modified epimorphin obtained by adding a
2 hydrophilic peptide composed of 5 to 99 amino acids to at
3 least one terminus of a polypeptide containing the
4 functional domain of epimorphin.

1 2. The modified epimorphin according to Claim 1,
2 wherein the functional domain of epimorphin is defined as
3 a central fragment (2) obtained by deleting a coiled coil
4 domain (1) on the N-terminal side, a coiled coil domain
5 (3) on the C-terminal side and a hydrophobic domain
6 adjacent to the C-terminus from the whole-length
7 epimorphin.

1 3. The modified epimorphin according to Claim 1 or
2 2, wherein the polypeptide containing the functional
3 domain of epimorphin is the whole-length epimorphin.

1 4. The modified epimorphin according to Claim 1 or
2 2, wherein the polypeptide containing the functional
3 domain of epimorphin is a fragment obtained by deleting
4 part of amino acids from the whole-length epimorphin from
5 the side of at least one terminus thereof.

1 5. The modified epimorphin according to Claim 4,
2 wherein the polypeptide containing the functional domain

3 of epimorphin is a fragment a obtained by deleting at
4 least part of amino acids in the coiled coil domain (1)
5 from the whole-length epimorphin from the N-terminal side
6 thereof.

1 6. The modified epimorphin according to Claim 4,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment b obtained by deleting the C-
4 terminal hydrophobic domain from the whole-length
5 epimorphin.

1 7. The modified epimorphin according to Claim 4,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment c obtained by deleting the C-
4 terminal hydrophobic domain from the whole-length
5 epimorphin and moreover deleting at least part of amino
6 acids in the coiled coil domain (1) thereof from the N-
7 terminal side.

1 8. The modified epimorphin according to Claim 4,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment d obtained by deleting the C-
4 terminal hydrophobic domain from the whole-length
5 epimorphin and moreover deleting at least part of amino
6 acids in the coiled coil domain (3) thereof from the C-
7 terminal side.

1 9. The modified epimorphin according to Claim 4,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment e obtained by deleting the C-
4 terminal hydrophobic domain from the whole-length
5 epimorphin and moreover deleting at least part of amino
6 acids in the coiled coil domain (1) thereof from the N-
7 terminal side and at least part of amino acids in the
8 coiled coil domain (3) thereof from the C-terminal side.

1 10. The modified epimorphin according to Claim 3,
2 wherein the hydrophilic peptide is added to both termini
3 of the whole-length epimorphin.

1 11. The modified epimorphin according to Claim 5,
2 wherein the hydrophilic peptide is added to at least the
3 N-terminus out of both termini of the fragment a.

1 12. The modified epimorphin according to Claim 6,
2 wherein the hydrophilic peptide is added to at least the
3 N-terminus out of both termini of the fragment b.

1 13. The modified epimorphin according to Claim 7,
2 wherein the hydrophilic peptide is added to at least the
3 N-terminus out of both termini of the fragment c.

1 14. The modified epimorphin according to Claim 8,
2 wherein the hydrophilic peptide is added to at least the

3 C-terminus out of both termini of the fragment d.

1 15. The modified epimorphin according to any one of
2 Claims 1 to 14, wherein the hydrophilic peptide contains
3 bound hydrophilic amino acids in a proportion of at least
4 50% based on the number of amino acids.

1 16. The modified epimorphin according to any one of
2 Claims 1 to 15, wherein the hydrophilic peptide contains
3 at least one amino acid sequence selected from the group
4 consisting of the following amino acid sequences (a) to
5 (h):

- 6 (a) Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala;
7 (b) Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu-Asn;
8 (c) Glu-Tyr-Lys-Glu-Glu-Glu-Glu-Lys;
9 (d) Tyr-Thr-Asp-Ile-Glu-Met-Asn-Arg-Leu-Gly-Lys;
10 (e) Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-
11 Ile-Gly-Lys;
12 (f) Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly-Arg;
13 (g) Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp; and
14 (h) Gly-Ala-Pro-Val-Pro-Tyr-Asp-Pro-Leu-Glu-Pro-Arg.

1 17. The modified epimorphin according to any one of
2 Claims 1 to 15, wherein the hydrophilic peptide contains
3 at least 5 consecutive histidine residues.

1 18. The modified epimorphin according to any one of

2 Claims 1 to 17, which has been subjected to intermolecular
3 crosslinking to form a complex.

1 19. The modified epimorphin according to any one of
2 Claims 1 to 18, wherein the epimorphin is human
3 epimorphin.

1 20. The modified epimorphin according to Claim 19,
2 wherein the human epimorphin is one selected from the
3 group consisting of epimorphin represented by SEQ ID NO.
4 1, epimorphin isoform A represented by SEQ ID NO. 2 and
5 epimorphin isoform B represented by SEQ ID NO. 3, all
6 shown in SEQUENCE TABLE.

1 21. The modified epimorphin according to Claim 19 or
2 20, wherein the polypeptide containing the functional
3 domain of epimorphin contains at least an amino acid
4 sequence ranging from the 99th amino acid to the 189th
5 amino acid from the N-terminus of the human epimorphin.

1 22. The modified epimorphin according to Claim 19 or
2 20, wherein the polypeptide containing the functional
3 domain of epimorphin contains at least an amino acid
4 sequence ranging from the 104th amino acid to the 187th
5 amino acid from the N-terminus of the human epimorphin.

1 23. The modified epimorphin according to Claim 19 or

2 20, wherein the polypeptide containing the functional
3 domain of epimorphin is a fragment obtained by deleting 1
4 to 28 amino acids in the coiled coil domain (1) from the
5 whole-length human epimorphin from the N-terminal side and
6 deleting the C-terminal hydrophobic domain therefrom.

1 24. The modified epimorphin according to Claim 19 or
2 20, wherein the polypeptide containing the functional
3 domain of epimorphin is a fragment obtained by deleting 29
4 to 103 amino acids from the whole-length human epimorphin
5 from the N-terminal side.

1 25. The modified epimorphin according to Claim 24,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment obtained by deleting 29 to 77
4 amino acids in the coiled coil domain (1) from the whole-
5 length human epimorphin from the N-terminal side and
6 deleting the C-terminal hydrophobic domain therefrom.

1 26. The modified epimorphin according to Claim 24,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment obtained by deleting 78 to 103
4 amino acids in the coiled coil domain (1) from the whole-
5 length human epimorphin from the N-terminal side and
6 deleting the C-terminal hydrophobic domain therefrom.

1 27. The modified epimorphin according to Claim 19 or

2 20, wherein the polypeptide containing the functional
3 domain of epimorphin is a fragment obtained by deleting 30
4 to 98 amino acids in the coiled coil domain (1) from the
5 whole-length human epimorphin from the N-terminal side and
6 deleting the C-terminal hydrophobic domain therefrom.

1 28. The modified epimorphin according to any one of
2 Claims 1 to 18, wherein the epimorphin is mouse
3 epimorphin.

1 29. The modified epimorphin according to Claim 28,
2 wherein the mouse epimorphin is one selected from the
3 group consisting of epimorphin represented by SEQ ID NO.
4 4, epimorphin isoform A represented by SEQ ID NO. 5 and
5 epimorphin isoform B represented by SEQ ID NO. 6, all
6 shown in SEQUENCE TABLE.

1 30. The modified epimorphin according to Claim 28 or
2 29, wherein the polypeptide containing the functional
3 domain of epimorphin contains at least an amino acid
4 sequence ranging from the 100th amino acid to the 190th
5 amino acid from the N-terminus of the mouse epimorphin.

1 31. The modified epimorphin according to Claim 28 or
2 29, wherein the polypeptide containing the functional
3 domain of epimorphin contains at least an amino acid
4 sequence ranging from the 105th amino acid to the 188th

5 amino acid from the N-terminus of the mouse epimorphin.

1 32. The modified epimorphin according to Claim 28 or
2 29, wherein the polypeptide containing the functional
3 domain of epimorphin is a fragment obtained by deleting 1
4 to 29 amino acids in the coiled coil domain (1) from the
5 whole-length mouse epimorphin from the N-terminal side and
6 deleting the C-terminal hydrophobic domain therefrom.

1 33. The modified epimorphin according to Claim 28 or
2 29, wherein the polypeptide containing the functional
3 domain of epimorphin is a fragment obtained by deleting 30
4 to 104 amino acids from the whole-length mouse epimorphin
5 from the N-terminal side.

1 34. The modified epimorphin according to Claim 33,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment obtained by deleting 30 to 78
4 amino acids in the coiled coil domain (1) from the whole-
5 length mouse epimorphin from the N-terminal side and
6 deleting the C-terminal hydrophobic domain therefrom.

1 35. The modified epimorphin according to Claim 33,
2 wherein the polypeptide containing the functional domain
3 of epimorphin is a fragment obtained by deleting 79 to 104
4 amino acids in the coiled coil domain (1) from the whole-
5 length mouse epimorphin from the N-terminal side and

6 deleting the C-terminal hydrophobic domain therefrom.

1 36. The modified epimorphin according to Claim 28 or
2 29, wherein the polypeptide containing the functional
3 domain of epimorphin is a fragment obtained by deleting 30
4 to 99 amino acids in the coiled coil domain (1) from the
5 whole-length mouse epimorphin from the N-terminal side and
6 deleting the C-terminal hydrophobic domain therefrom.

1 37. A modified epimorphin composed of a polypeptide
2 having a structure that a hydrophobic domain adjacent to
3 the C-terminus of the whole-length epimorphin consisting
4 of a coiled coil domain (1) on the N-terminal side, a
5 functional domain (2) at the center, a coiled coil domain
6 (3) on the C-terminal side and the hydrophobic domain (4)
7 adjacent to the C-terminus has been deleted from the
8 whole-length epimorphin, and at least part of amino acids
9 have been deleted from the terminal side of at least one
10 of the coiled coil domains (1) and (3) as well.

1 38. The modified epimorphin according to Claim 37,
2 which is composed of a polypeptide having a structure that
3 at least part of amino acids of the coiled coil domain (1)
4 have been deleted from the N-terminal side.

1 39. The modified epimorphin according to Claim 37 or
2 38, wherein the epimorphin is human epimorphin.

1 40. The modified epimorphin according to Claim 39,
2 which is composed of a polypeptide having a structure that
3 1 to 28 amino acids have been deleted form the N-terminal
4 side of the coiled coil domain (1).

1 41. The modified epimorphin according to Claim 39,
2 which is composed of a polypeptide having a structure that
3 29 to 77 amino acids have been deleted form the N-terminal
4 side of the coiled coil domain (1).

1 42. The modified epimorphin according to Claim 39,
2 which is composed of a polypeptide having a structure that
3 78 to 103 amino acids have been deleted form the N-
4 terminal side of the coiled coil domain (1).

1 43. The modified epimorphin according to Claim 39,
2 which is a human modified epimorphin represented by any
3 one of amino acid sequences of SEQ ID NO. 15 to 17 shown
4 in SEQUENCE TABLE.

1 44. The modified epimorphin according to Claim 37 or
2 38, wherein the epimorphin is mouse epimorphin.

1 45. The modified epimorphin according to Claim 44,
2 which is composed of a polypeptide having a structure that
3 1 to 29 amino acids have been deleted form the N-terminal
4 side of the coiled coil domain (1).

1 46. The modified epimorphin according to Claim 44,
2 which is composed of a polypeptide having a structure that
3 30 to 78 amino acids have been deleted form the N-terminal
4 side of the coiled coil domain (1).

1 47. The modified epimorphin according to Claim 44,
2 which is composed of a polypeptide having a structure that
3 79 to 104 amino acids have been deleted form the N-
4 terminal side of the coiled coil domain (1).

1 48. The modified epimorphin according to Claim 44,
2 which is a mouse modified epimorphin represented by any
3 one of amino acid sequences of SEQ ID NO. 18 to 20 shown
4 in SEQUENCE TABLE.

1 49. DNA encoding the modified epimorphin according
2 to any one of Claims 1 to 48.

1 50. The DNA according to Claim 49, wherein the
2 modified epimorphin is one according to any one of Claims
3 1 to 36.

1 51. The DNA according to Claim 49, wherein the
2 modified epimorphin is one according to any one of Claims
3 37 to 48.

1 52. The DNA according to Claim 51, which is DNA

2 encoding a human modified epimorphin represented by any
3 one of base sequences of SEQ ID NOs. 7 to 9 shown in
4 SEQUENCE TABLE.

1 53. The DNA according to Claim 51, which is DNA
2 encoding a mouse modified epimorphin represented by any
3 one of base sequences of SEQ ID NOs. 11 to 13 shown in
4 SEQUENCE TABLE.

1 54. A recombinant vector which contains DNA encoding
2 the modified epimorphin according to any one of Claims 1
3 to 48 and is capable of expressing its corresponding
4 polypeptide.

1 55. The recombinant vector according to Claim 54,
2 wherein the DNA is DNA encoding the modified epimorphin
3 according to any one of Claims 1 to 36.

1 56. The recombinant vector according to Claim 54,
2 wherein the DNA is DNA encoding the modified epimorphin
3 according to any one of Claims 37 to 48.

1 57. The recombinant vector according to Claim 56,
2 which contains DNA encoding a human modified epimorphin
3 represented by any one of base sequences of SEQ ID NOs. 7
4 to 9 shown in SEQUENCE TABLE, and is capable of expressing
5 its corresponding polypeptide.

1 58. The recombinant vector according to Claim 56,
2 which contains DNA encoding a mouse modified epimorphin
3 represented by any one of base sequences of SEQ ID NOs. 11
4 to 13 shown in SEQUENCE TABLE, and is capable of
5 expressing its corresponding polypeptide.

1 59. A transformant obtained by introducing a
2 recombinant vector which contains DNA encoding the
3 modified epimorphin according to any one of Claims 1 to 48
4 and is capable of expressing its corresponding
5 polypeptide.

1 60. The transformant according to Claim 59, wherein
2 the recombinant vector contains DNA encoding the modified
3 epimorphin according to any one of Claims 1 to 36.

1 61. The transformant according to Claim 59, wherein
2 the recombinant vector contains DNA encoding the modified
3 epimorphin according to any one of Claims 37 to 48.

1 62. The transformant according to Claim 61, which is
2 obtained by introducing a recombinant vector containing
3 DNA encoding a human modified epimorphin represented by
4 any one of base sequences of SEQ ID NOs. 7 to 9 shown in
5 SEQUENCE TABLE, said vector being capable of expressing
6 its corresponding polypeptide.

1 63. The transformant according to Claim 61, which is
2 obtained by introducing a recombinant vector containing
3 DNA encoding a mouse modified epimorphin represented by
4 any one of base sequences of SEQ ID NOs. 11 to 13 shown in
5 SEQUENCE TABLE, said vector being capable of expressing
6 its corresponding polypeptide.

1 64. The transformant according to any one of Claims
2 59 to 63, wherein a host of the transformant is
3 Escherichia coli.

1 65. A method of producing a modified epimorphin,
2 comprising using a transformant obtained by introducing a
3 recombinant vector which contains DNA encoding the
4 modified epimorphin according to any one of Claims 1 to 48
5 and is capable of expressing its corresponding
6 polypeptide.

1 66. The method according to Claim 65, wherein the
2 transformant is obtained by introducing a recombinant
3 vector which contains DNA encoding the modified epimorphin
4 according to any one of Claims 1 to 36 and is capable of
5 expressing its corresponding polypeptide.

1 67. The method according to Claim 65, wherein the
2 transformant is obtained by introducing a recombinant
3 vector which contains DNA encoding the modified epimorphin

4 according to any one of Claims 37 to 48 and is capable of
5 expressing its corresponding polypeptide.

1 68. The method according to Claim 67 for producing a
2 human modified epimorphin, which comprises using a
3 transformant obtained by introducing a recombinant vector
4 containing DNA encoding a human modified epimorphin
5 represented by any one of base sequences of SEQ ID NOS. 7
6 to 9 shown in SEQUENCE TABLE, said vector being capable of
7 expressing its corresponding polypeptide.

1 69. The method according to Claim 67 for producing a
2 mouse modified epimorphin, which comprises using a
3 transformant obtained by introducing a recombinant vector
4 containing DNA encoding a human modified epimorphin
5 represented by any one of base sequences of SEQ ID NOS. 11
6 to 13 shown in SEQUENCE TABLE, said vector being capable
7 of expressing its corresponding polypeptide.

1 70. A variant modified epimorphin obtained by making
2 partial substitution, deletion and/or insertion of amino
3 acids in the amino acid sequence of the modified
4 epimorphin according to any one of Claims 1 to 48, wherein
5 the variant maintains the function of the original
6 sequence.

1 71. The variant modified epimorphin according to

2 Claim 70, wherein the site of the amino acid sequence, at
3 which the partial substitution, deletion or insertion of
4 amino acids is made in the amino acid sequence of the
5 modified epimorphin, is in the amino acid sequence of a
6 polypeptide containing the functional domain of
7 epimorphin.

1 72. The variant modified epimorphin according to
2 Claim 70, wherein the site of the amino acid sequence, at
3 which the partial substitution, deletion or insertion of
4 amino acids is made in the amino acid sequence of the
5 modified epimorphin, is in the amino acid sequence of the
6 functional domain of epimorphin.

1 73. DNA encoding the variant modified epimorphin
2 according to any one of Claims 70 to 72.

1 74. A recombinant vector which contains DNA encoding
2 the variant modified epimorphin according to Claim 73, and
3 is capable of expressing its corresponding polypeptide.

1 75. A transformant obtained by introducing the
2 recombinant vector according to Claim 74.

1 76. A method of producing a variant modified
2 epimorphin, comprising using the transformant according to
3 Claim 75.

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Fig. 1

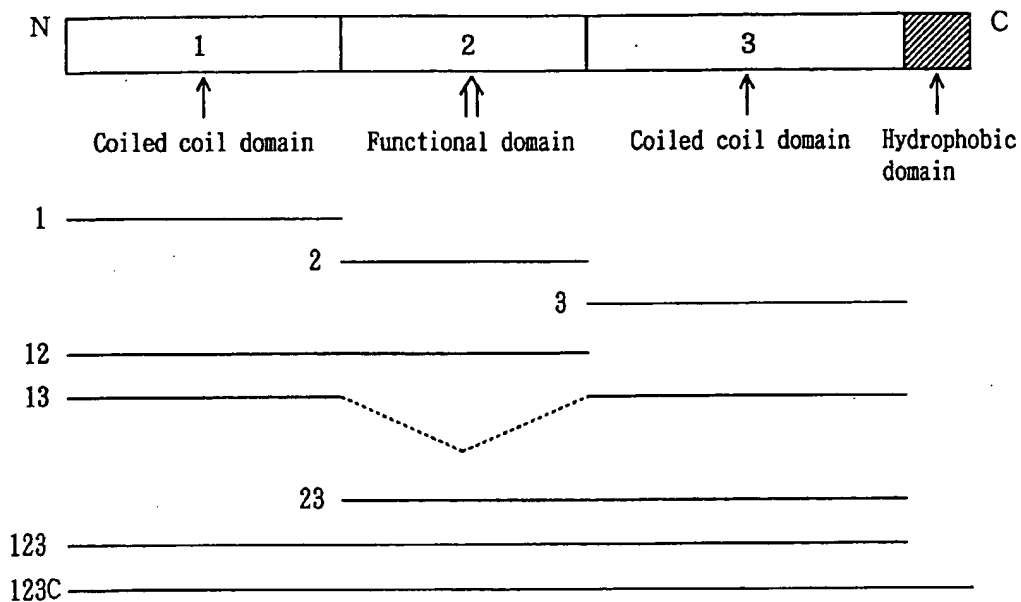
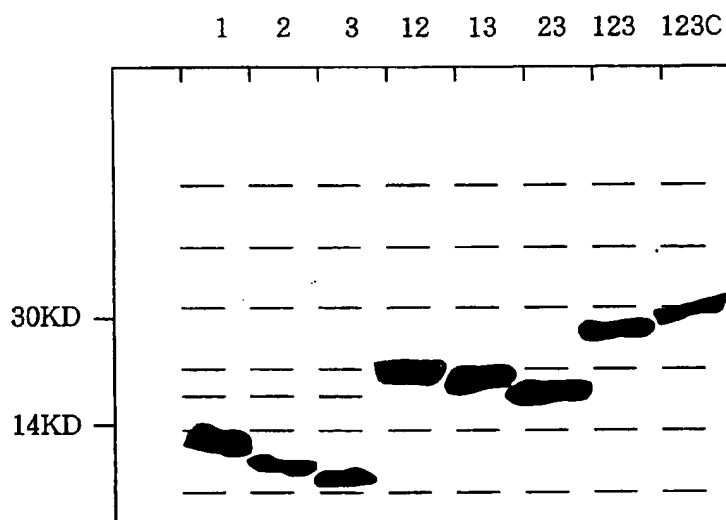


Fig. 2



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Fig. 3

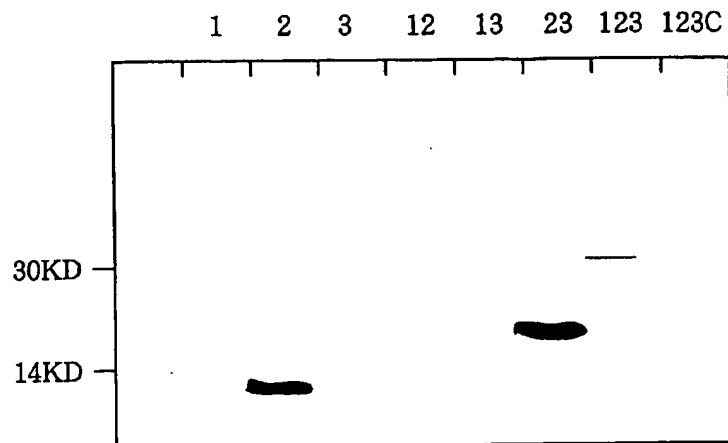


Fig. 4

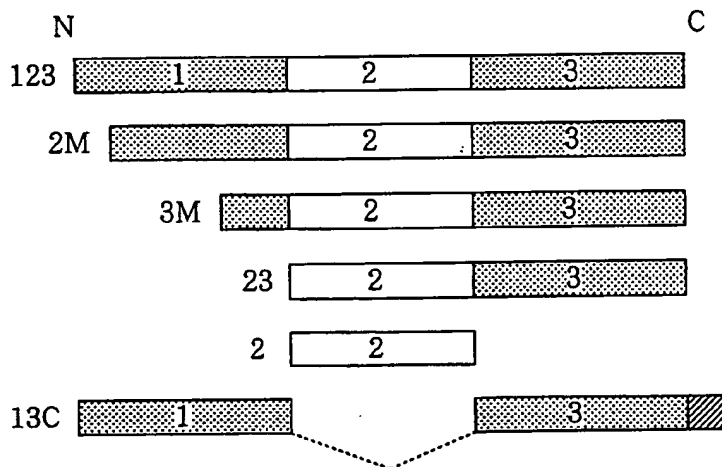


Fig. 5

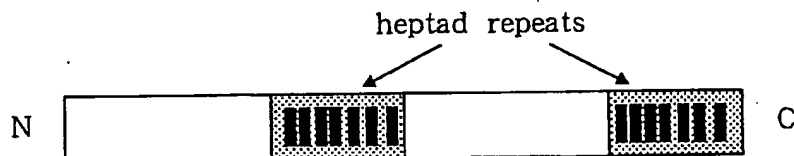


Fig. 6

